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FOREWORD

In 1982 *Escherichia coli* was used to commercially produce the first biopharmaceutical, recombinant human insulin. Since then the biopharmaceutical industry has steadily grown and currently produces over 200 biopharmaceutical proteins using bacterial, mammalian and yeast cell culture systems. With the maturation of the biopharmaceutical industry from research and development to commercial biomanufacture, biomanufacturing education and training programs have sprung up around mature biotechnology clusters throughout the nation and globe to support them. The Northeast Biomanufacturing Center and Collaborative (NBC^2) has developed a Global Biomanufacturing Curriculum (GBC) that includes this hands-on laboratory manual that provides Standard Operating Procedures (SOPs) for the core jobs of cell culture, purification, quality control and quality assurance. The manual also includes education and training in metrology, validation, and environmental health and safety.[1] An industry written textbook will be published in January 2010. Ancillaries such as an instructor's manual, power point slide shows, and virtual laboratories to support the hands-on laboratories are under development.

This hands-on laboratory manual is intended for use in biotechnology/biomanufacturing education and training programs. It is applicable to both educational and industrial settings. It is effective in introducing students to the regulatory atmosphere of the industry or current Good Manufacturing Practices (cGMPs) and to developing skills in upstream and downstream processing, quality control microbiology and biochemistry, and quality assurance.

Core production systems (cell culture, purification and quality control biochemistry) for three proteins are included. The core production systems can be used to produce, purify, sample and test recombinant jellyfish green fluorescent protein (GFP) and two human recombinant proteins, tissue plasminogen activator (tPA) and human serum albumin (HSA).

Construction of the Standard Operating Procedures (SOPs) and Batch Records in this manual began in 1994 with a grant from the National Science Foundation Advanced

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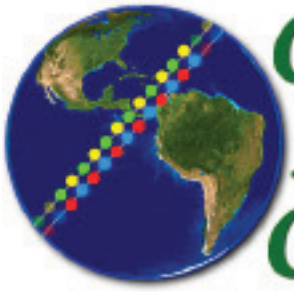
[1] It should be noted that while SOPs for specific models of laboratory equipment are included in this manual that the procedures can be carried out with alternative models. If alternate models are used, we suggest the instructor (or students) write SOPs for the specific equipment in their laboratory. Also, it is not necessary to use a bioreactor for upstream processing of recombinant proteins. Alternative protocols for producing tPA and HSA in a spinner or shake flask are located in the Appendix.

Technological Education program's first solicitation in 1993. Many of the documents in this book were originally developed by Dr. Sonia Wallman, the Principal Investigator of the NSF ATE grant awarded in 1994. Since then many people have contributed to the continuing development of these SOPs, including instructors, staff, and students in the Biotechnology/Biomanufacturing programs at Great Bay Community College, Montgomery County Community College, Finger Lakes Community College, the Community College of Rhode Island, Baltimore County Community College and Minuteman Regional High School.

We wish to recognize the National Science Foundation's Advanced Technological Education Program (NSF #0501953) that supported the development of this set of Standard Operating Procedures for biomanufacturing technician education and training and for rewarding careers in biomanufacturing.

Sonia Wallman, Ph.D.
Director, Northeast Biomanufacturing Center and Collaborative (NBC^2)
July 2009

Metrology



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- **Label:** Calibration Not Required.....35
- **Competencies List:** Maintenance/Instrumentation Technician.....37

Great Bay Community College
320 Corporate Drive
Portsmouth, NH 03801

Title: Scout® *Pro* Balance Operation

Approvals:
Preparer: __ Bob O'Brien _____ Date _19Jun08_____
Reviewer: __ Deb Audino_____ Date _19Jun08_____

1. **Purpose:**
   1.1. Operation of Scout® *Pro* Balance.
2. **Scope:**
   2.1. Applies to the weight measurement of laboratory materials.
3. **Responsibilities:**
   3.1 .It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2.It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Scout® *Pro* Balance Instruction Manual.
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. Appropriate weighing container(s) such as weigh boat, weigh paper, beaker, flask, bottle, etc.
8. **Procedure:**
   8.1. Turn the scale on using the ON/ZERO button (Figure 1).
   8.2. Select weight unit by holding down the PRINT button (Figure 1) and scrolling through the options. Release the PRINT button when desired unit is displayed (Figure 1).
   8.3. Place weighing container on the balance weight pan (Figure 1). Press the ON/ZERO button to tare the scale.
   Note: Standard mass weights can be placed directly on the balance weight pan. If the displayed value for the standard mass weight is greater than ±0.01g of the standard weight, then the scale needs to be calibrated. Refer to the Scout® *Pro* Balance Calibration SOP for proper calibration of the balance.
   8.4. Load weighing container with material to be weighed. When the scale has reached a stable reading, the stability indicator (star) will appear on the bottom left corner of the display next to the weight.
   8.5. To turn balance off, press and hold the ON/ZERO button for at least 3 seconds.
9. **Attachments:**
   9.1. Figure 1: Scout® *Pro* Balance
10. **History:**

Name	Date	Amendment
Ellery Raitt	04/18/05	Initial Release
Bob O'Brien	14Apr07	Updated the date format and re-labeled the photo .
Bob O'Brien	20Jun08	College name change

Title: Scout® *Pro* Balance Operation

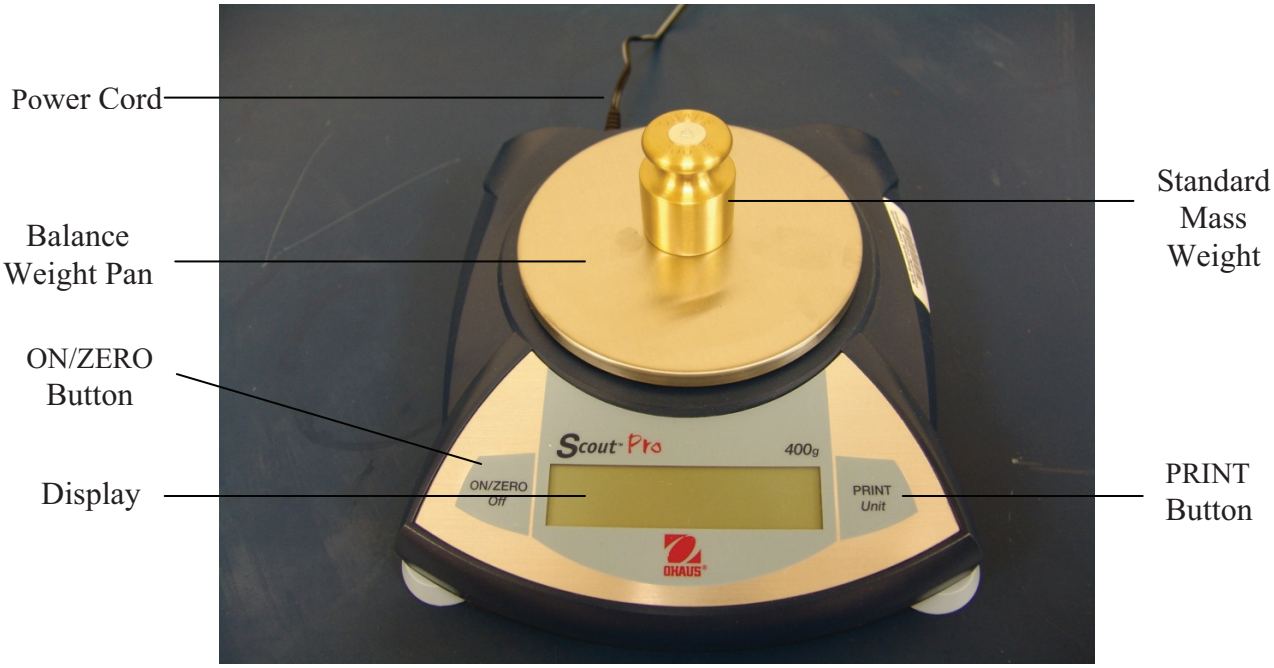

Figure 1: Scout® *Pro* Balance

Title: Scout® *Pro* Balance Calibration

Approvals:
Preparer: Bob O'Brien_____ Date_19Jun08_____
Reviewer: Deb Audino_____ Date_19Jun08_____

1. **Purpose:**
   1.1. Calibration of Scout® *Pro* Balance.
2. **Scope:**
   2.1. Applies to the calibration of Scout® *Pro* Balance.
3. **Responsibilities:**
   3.1 .It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2.It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Scout® *Pro* Balance Instruction Manual
   4.2 Scout® *Pro* Balance Operation SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. 20g standard mass
   7.2. 100g standard mass
   7.3. 200g standard mass
   7.4. Scout® *Pro* Balance
8. **Procedure:**
   8.1. **Span Calibration**
      8.1.1. Place the Lock switch (on bottom side of balance) to the un-lock position.
      8.1.2. Verify that the weighing platform is clear.
      8.1.3. Press and hold the ON/ZERO button (Figure 1) for at least 5 seconds. When the display reads "MENU" release the button. The display should now read ".C.A.L.".
      8.1.4. Press the ON/ZERO button to acquire the zero weight reading. The display will flash "-C-".
      8.1.5. The display will automatically change to flash "200.00". Place the 200g standard mass weight on the platform. Press the ON/ZERO button.
      8.1.6. When the scale had finished calibrating, the display will read "donE".
      8.1.7. Remove standard weight. The display should read 0.00. Scale Tolerance for span calibration is ± 0.01g.
      8.1.8. For quick calibration purposes validate the balance by weighing 20g and 200g three times each. Make sure the deviation is within the scale tolerance of ± 0.01g.
   8.2. **Linear Calibration**
      8.2.1. Make sure the weighing platform is clear.

Title: Scout® *Pro* Balance Calibration

8.2.2. Starting with the balance off, press and hold the ON/ZERO button (Figure 1) for at least 5 seconds. When the display reads "MENU" release the button. The display should now read "CAL".
8.2.3. Press the PRINT UNIT button (Figure 1). The display should read "SETUP".
8.2.4. Press the ON/ZERO button. The display should read "A-OFF".
8.2.5. Press the PRINT UNIT button. The display should read "L in".
8.2.6. Press the ON/ZERO button. The display will flash "-C-"momentarily, followed by "100g".
8.2.7. Place the 100g standard mass weight on the platform. Press the ON/ZERO button and the display will flash "-C-"momentarily followed "by 200g".
8.2.8. Remove the 100g standard mass weight and place a 200g standard mass weight on the platform. Press the ON/ZERO button and the display will flash "-C-"followed by "donE".
8.2.9. Remove the 200g standard mass weight and the display should read "0.00". Scale tolerance for linear calibration is ± 0.01g.
8.2.10. For quick calibration purposes validate the balance by weighing 20g and 200g three times respectively. Make sure the deviation is within the scale tolerance of ± 0.01g.

9. Attachments:

9.1. Figure 1: Scout® *Pro* Balance

10. History:

Name	Date	Amendment
Marlo Austria	04Nov05	Initial release
Bob O'Brien	02Jun06	Added photo.
Bob O'Brien	12Feb07	Updated date format.
Bob O'Brien	19Jun08	College name change

Title: Scout® *Pro* Balance Calibration

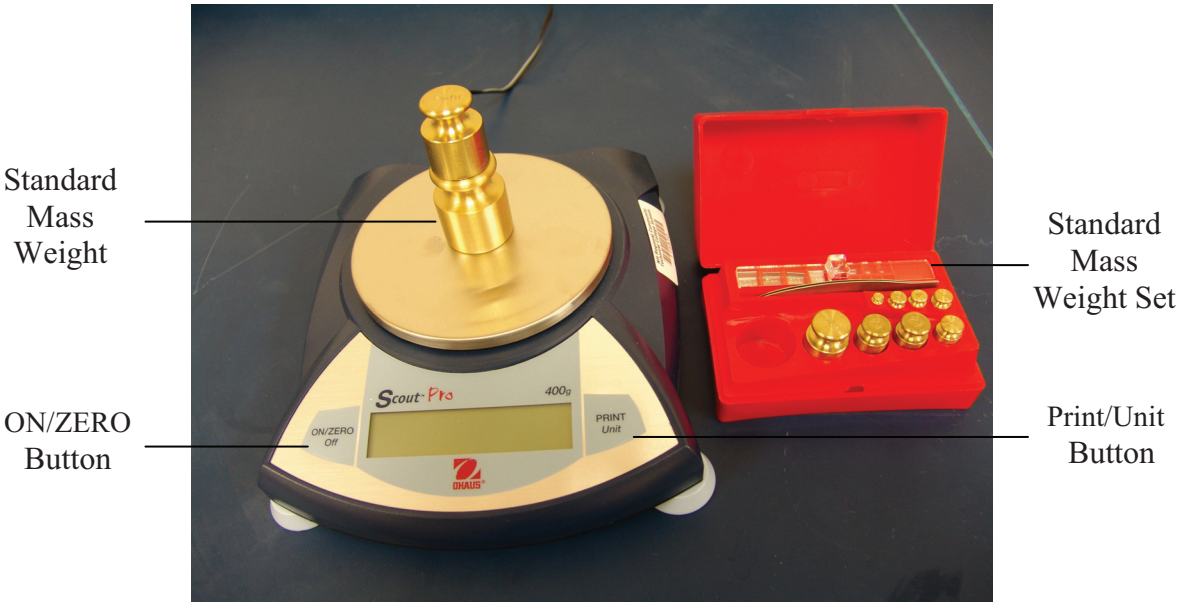

Figure 1: Scout® *Pro* Balance

Balance Calibration Form

Balance Information

Name and Description: _____

Model: _____

Serial Number:_____

Calibration Information

Date of Calibration: _____ **Technician:**_____

Standard Mass Weights Used:_____

Comments:_____

Passed Calibration:	**Failed Calibration:**
Pass: □Yes □ No Calibration Sticker: □Yes □ No □ Not Applicable Next Calibration Due Date:_____	Reason for Failure:_____ Date Out of Service:_____ Initials:_____

History:

Name	Date	Amendment
Bob O'Brien	07Jun07	Initial Release
Bob O'Brien	19Aug08	College name change
Kari Britt	29Oct09	Changed name of form from Calibration Form to Balance Calibration Form.

Title: Orion 4 Star pH Meter SOP

Approvals:
Preparer: _____ Bob O'Brien _____ Date __18Jul08_____
Reviewer: ____ Deb Audino_____ Date__18Jul08_____

1. **Purpose:**
   1.1. To calibrate and operate the Orion Star Series pH Meter.
2. **Scope:**
   2.1. To measure the pH of solutions and media.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Thermo Scientific Orion Star Series pH Meter Quick Start Guide
   4.2. Orion Star Series Meter Abridged User's Guide
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Use caution when handling all samples due to unknown pH.
   6.2. Do not wipe or rub electrode. This will create a static build up that will interfere with measurements.
   6.3. Always wear the appropriate personal protective equipment (PPE).
7. **Materials:**
   7.1. Orion Star Series pH Meter
   7.2. Triode 3 in 1 pH ATC, expoxy body electrode
   7.3. reference electrode filling solution (Orion 900011)
   7.4. pH electrode storage solution
   7.5. commercially made pH standard buffers as required
   7.6. wash bottle
   7.7. deionized (DI) water
   7.8. waste beaker
   7.9. laboratory tissues, such as Kimwipes
8. **Procedure:**
   8.1. **Preparation**
      8.1.1. Select the pH 7 standard buffer and a second standard buffer that brackets the expected sample pH.
      8.1.2. Prepare buffers according to manufacturer's instructions if needed, and ensure that they are not expired.
   8.2. **Calibration**
      8.2.1. Press the power key to turn on the pH meter.
      8.2.2. Gently remove the protective cap/sleeve from the bottom of the electrode.
      8.2.3. Unplug the blue cap from the fill hole on the electrode.

Title: Orion 4 Star pH Meter SOP

8.2.4. If necessary, fill the electrode with the filling solution until the solution is about 0.5cm below the fill hole.
8.2.5. Rinse the electrode with DI water and gently blot dry with a laboratory tissue.
8.2.6. Press the calibrate key (pH/Cal will be displayed).
8.2.7. Insert the electrode into pH 7 standard buffer.
8.2.8. Wait for the pH icon to stop flashing and the ► to start flashing.
8.2.9. Press the calibrate key (Cal.2 is displayed).
8.2.10. Remove the electrode from the solution, rinse the electrode with DI water and blot dry with a laboratory tissue.
8.2.11. Insert electrode into the second standard buffer.
8.2.12. Wait for the pH icon to stop flashing and the ► to start flashing.
8.2.13. Press the calibrate key and then press measure to save and end calibration.
8.2.14. SLP will be displayed. The acceptable range for the slope is 92-102%. Recalibrate if the slope is outside this range. Record the SLP value in the log book.
8.2.15. Verify that the meter is in the continuous mode as demonstrated by only the electrode icon displayed in the upper right corner of the display screen. If "AR" also appears, contact the instructor.
8.2.16. Remove the electrode from the solution, rinse the electrode with DI water and blot dry with a laboratory tissue.
8.2.17. Replace storage cap if immediate measurement of sample is not needed.

8.3. **Measurement**

8.3.1. If necessary, gently remove the protective cap/sleeve from the bottom of the electrode, rinse the electrode with DI water and blot dry with laboratory tissue.
8.3.2. Insert electrode into the sample.
8.3.3. Read pH value when the pH icon has stopped flashing for 5-10 seconds.
8.3.4. Remove the electrode from the solution, rinse the electrode with DI water and blot dry with a laboratory tissue.
8.3.5. Repeat steps 8.3.2. through 8.3.4. for additional samples.
8.3.6. When finished, replace protective cap onto end of electrode, replace the blue plug on the electrode, and turn off the pH meter.

9. Attachments:

9.1. Figure 1: Orion 4 star pH Meter Control Keys
9.2. Figure 2: pH Meter and Other Required Materials

10. History:

Name	Date	Amendment
Hazelgene Turner	21Sep07	Initial release
Bob O'Brien	18Jul08	Updated format. College name change

Title: Orion 4 Star pH Meter SOP

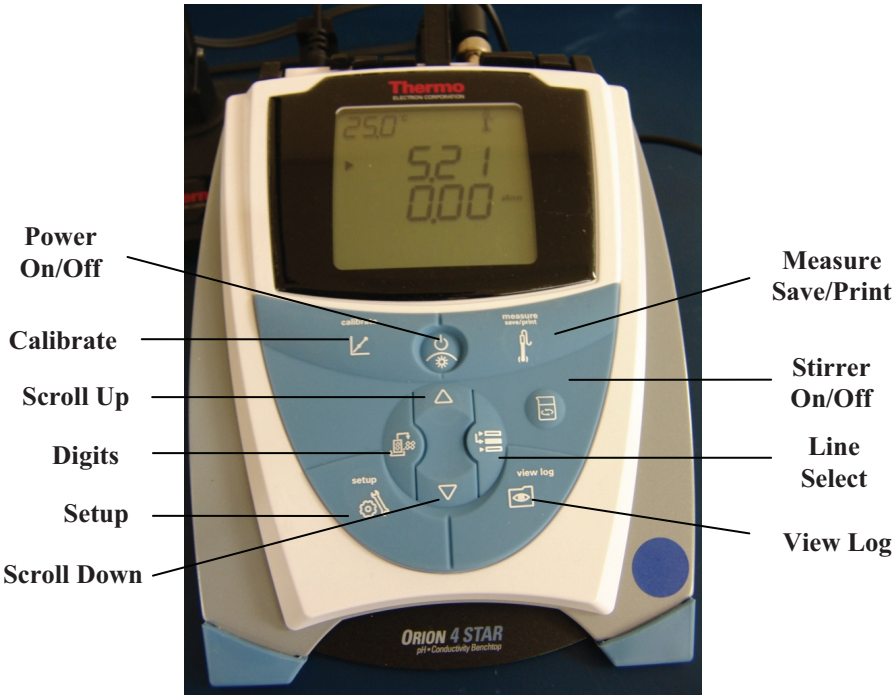

Figure 1: Orion 4 star pH Meter control keys

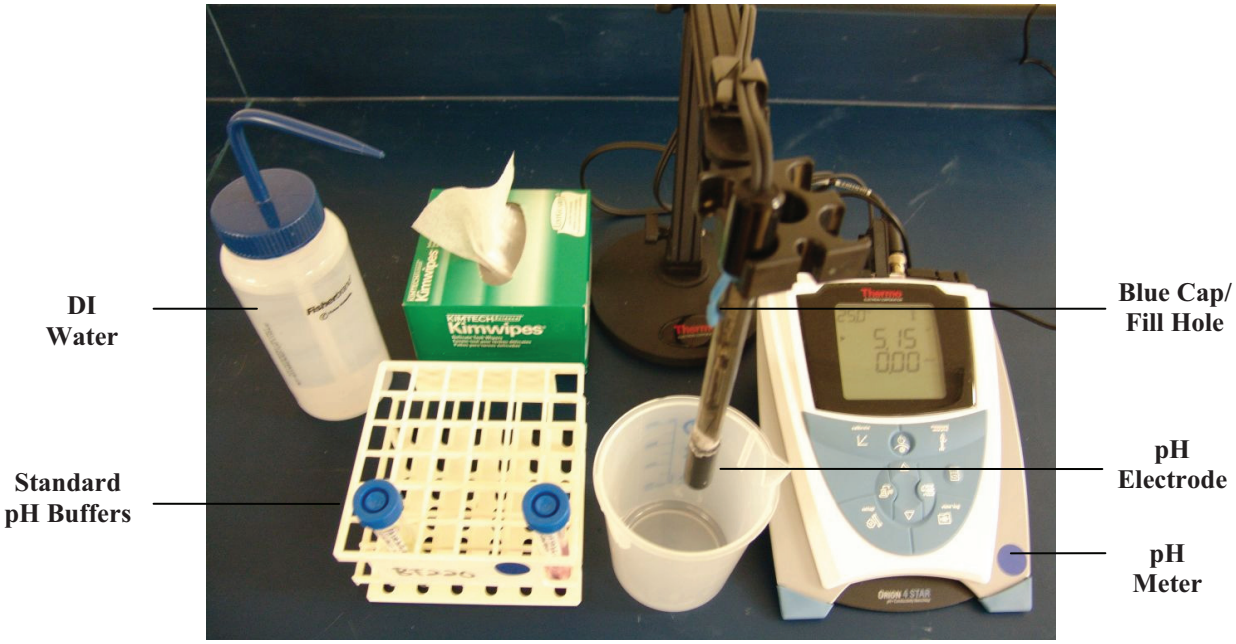

Figure 2: pH Meter and Other Required Materials

Title: Gilson Pipetman® P-20 to P-1000 Operation and Maintenance

Approvals:
Preparer: Bob O'Brien _____ Date _02Jun08_____
Reviewer: Deb Audino_____ Date _02Jun08_____

1. **Purpose:**
   Operation of Gilson Pipetman® P-20 through to the P-1000.
2. **Scope:**
   Applies to the operation, cleaning, and trouble shooting of the Gilson Pipetman®, designed to dispense precise volumes of liquid safely.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Gilson Pipetman® P users guide
   4.2. autoclave SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Volatile solutions: you should saturate the air-cushion of your pipette by aspirating and dispensing the solvent repeatedly before aspirating the sample.
   6.2. Acids or other corrosive liquids that emit vapors; can damage pipette to avoid this remove the tip holder and rinse the piston and O-ring and seal with distilled water.
   6.3. Temperature extremes can damage the Pipetman. Do not pipette liquids having temperatures of above 70^0C or below 4^0C.
7. **Materials:**
   7.1. Pipetman®
   7.2. pipette tips
   7.3. beaker
   7.4. weigh boats
   7.5. distilled water
   7.6. deionized water (DI water)
   7.7. lab towels
   7.8. 70% isopropyl alcohol (IPA)
   7.9. autoclave
8. **Procedure:**
   8.1. **Operation**
      8.1.1. The volume of liquid to be aspirated is set using the volumemeter which is read top (most significant digit) to bottom (least significant digit).
      8.1.2. Adjust the thumbwheel or push button to the desired volume. To obtain the maximum accuracy when setting the volume, set the volume 1/3 of a turn above the desired volume and then turn down to the desired volume.

Title: Gilson Pipetman® P-20 to P-1000 Operation and Maintenance

8.1.3. Double check that the set volume is correct while holding the volumemeter at eye level.
8.1.4. Fit a tip into the tip holder, by using a slight twisting motion when pressing the Pipetman® tip holder into a pipette tip to ensure a firm and airtight seal.
8.1.5. Follow direction below to pre-rinse the tip by aspirating the first volume of liquid and then dispensing it back into the sample container or a waste container.
8.1.6. Aspirate by pressing the push button to the first stop (Figure 1). Make sure that you operate the pushbutton slowly and smoothly.
8.1.7. Hold the pipette vertically and immerse the tip into the liquid hold at a constant depth just below the surface of the liquid (Table 1).
8.1.8. Dispense by placing the tip against the inside wall of the recipient vessel at an angle of 10° to 40° and then pressing the pushbutton slowly and smoothly to the first stop. Wait for at least one second; then press the pushbutton slowly and smoothly to second stop to expel any residual liquid from the tip.
8.1.9. Keep pushbutton completely depressed while removing the pipette tip from the vessel. Draw up the tip along inside surface of the vessel.
8.1.10. Release the pushbutton slowly and smoothly.

8.2. **Tip removal**

8.2.1. Tip may now be ejected by pressing firmly on the tip ejector button into a waste container.
8.2.2. Tip changes are required only if aspirating a different liquid, sample or reagent or volume. Tips should also be changed if aseptic technique is compromised (e.g. if the tip touches the outside of a container).
8.2.3. When you are finished pipetting, re-set the volume of the Pipetman® to the maximum volume for proper storage.

8.3. **Leak testing P20 – P200**

8.3.1. Fit a tip onto the Pipetman®.
8.3.2. Set the Pipetman® to the maximum volume given in the specification.
8.3.3. Using deionized water, pre-rinse the tip and then aspirate the set volume.
8.3.4. Hold the Pipetman® in a vertical position for approximately twenty seconds, while observing the end of the Pipetman® tip.
8.3.5. The water in pipette tip should remain constant.
8.3.6. If a droplet appears at the end of the tip there is a leak.
8.3.7. Verify that the tip is on tightly, and repeat the test.
8.3.8. If a droplet appears at the end of the tip there is a leak and the Pipetman® needs repair.

8.4. **Cleaning externally**

8.4.1. The Pipetman® is designed so that the parts that normally come in contact with liquid contaminants can easily be cleaned and decontaminated.
8.4.2. Wipe the outside of the entire Pipetman® with a lab towel dampened with a mild detergent solution.
8.4.3. Wipe the entire Pipetman® with a lab towel dampened with distilled water.
8.4.4. Remove the tip ejector.

Title: Gilson Pipetman® P-20 to P-1000 Operation and Maintenance

8.4.5. Wipe the tip ejector with a lab towel dampened with a mild soap solution.
8.4.6. Wipe the tip ejector with a lab towel dampened with distilled water.
8.4.7. Refit the tip ejector and allow the pipette to dry.
8.4.8. Dispose all used lab towels in a biohazard waste container.

8.5. **Immersion decontamination**

8.5.1. The following components: tip ejector, tip holder, connecting nut and the metal and plastic components of the piston assembly can be removed and immersed in IPA for complete decontamination. See Figure 2 and disassemble instructions below.
8.5.2. Allow the components to dry prior to reassembly. See directions below for reassembly.

8.6. **Chemical decontamination**

8.6.1. Spray a lab towel with 70% IPA to dampen.
8.6.2. Wipe upper part of body with dampened lab towel.
8.6.3. Wipe tip holder and tip ejector with dampened lab towel.
8.6.4. Wipe entire Pipetman® with a lab towel dampened with DI water.
8.6.5. Leave Pipetman® to dry or wipe dry with lab towel.
8.6.6. Dispose all used lab towels in a biohazard waste container.

8.7. **Trouble shooting**

8.7.1. See the trouble shooting table (Table 2).

8.8. **Disassemble the Pipetman®** (See Figure 2)

8.8.1. Remove tip ejector, Press the tip ejector button down and pull on the flanged upper part of the tip ejector with the other hand (moderate force may be required).
8.8.2. Remove the connecting nut by turning it counterclockwise direction by hand.
8.8.3. At this time carefully separate the lower components from the upper body assembly.
8.8.4. Remove the piston assembly and the o-ring and seal.
8.8.5. Clean the Pipetman® by following the decontamination instruction above.
8.8.6. Replace the necessary components as needed.

8.9. **Reassemble the Pipetman®**

8.9.1. Place fully assembled piston assembly into the tip holder.
8.9.2. Place connecting nut so that it can be pulled up over tip holder.
8.9.3. Holding the tip holder with connecting nut, line up and place the top of the piston assembly into the pipette upper body.
8.9.4. Tighten the connecting nut onto threads of upper pipette body.
8.9.5. Place tip ejector so it comes up over tip holder. Line up with tip ejector button rod in upper body.
8.9.6. Pull on the tip ejector flang with one hand with the other hand holding the upper body (moderate force may be required).

9. Attachments:

9.1. Figure 1: Pipetman®,
9.2. Figure 2: Pipetman® Component Breakout
9.3. Table 1: Immersion chart

Title: Gilson Pipetman® P-20 to P-1000 Operation and Maintenance

9.4. Table 2: Troubleshooting guide.

10. History:

Name	Date	Amendment
Bob O'Brien	08162006	Initial release
Bob O'Brien	02Jun08	Re-worked photo. Added re-assembly. College name change

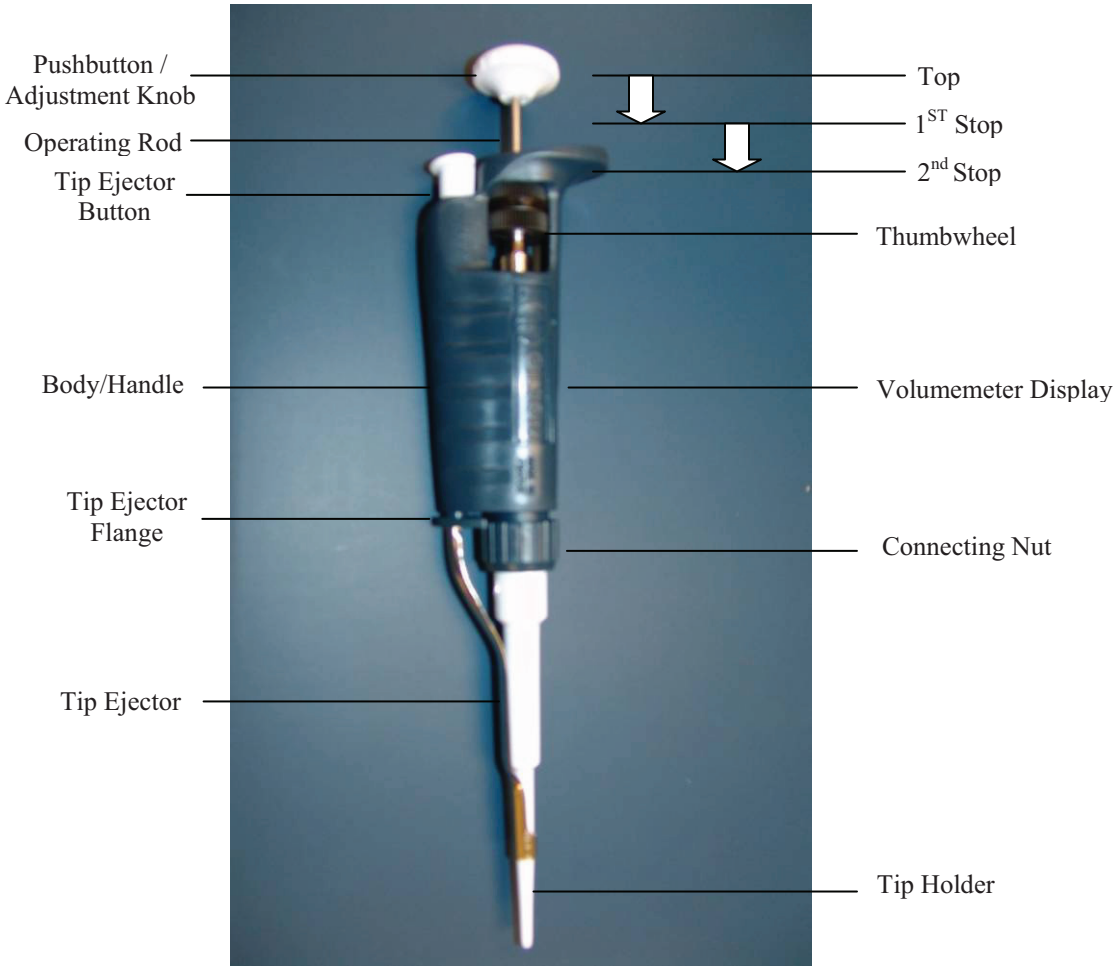

Figure 1: Pipetman®

Title: Gilson Pipetman® P-20 to P-1000 Operation and Maintenance

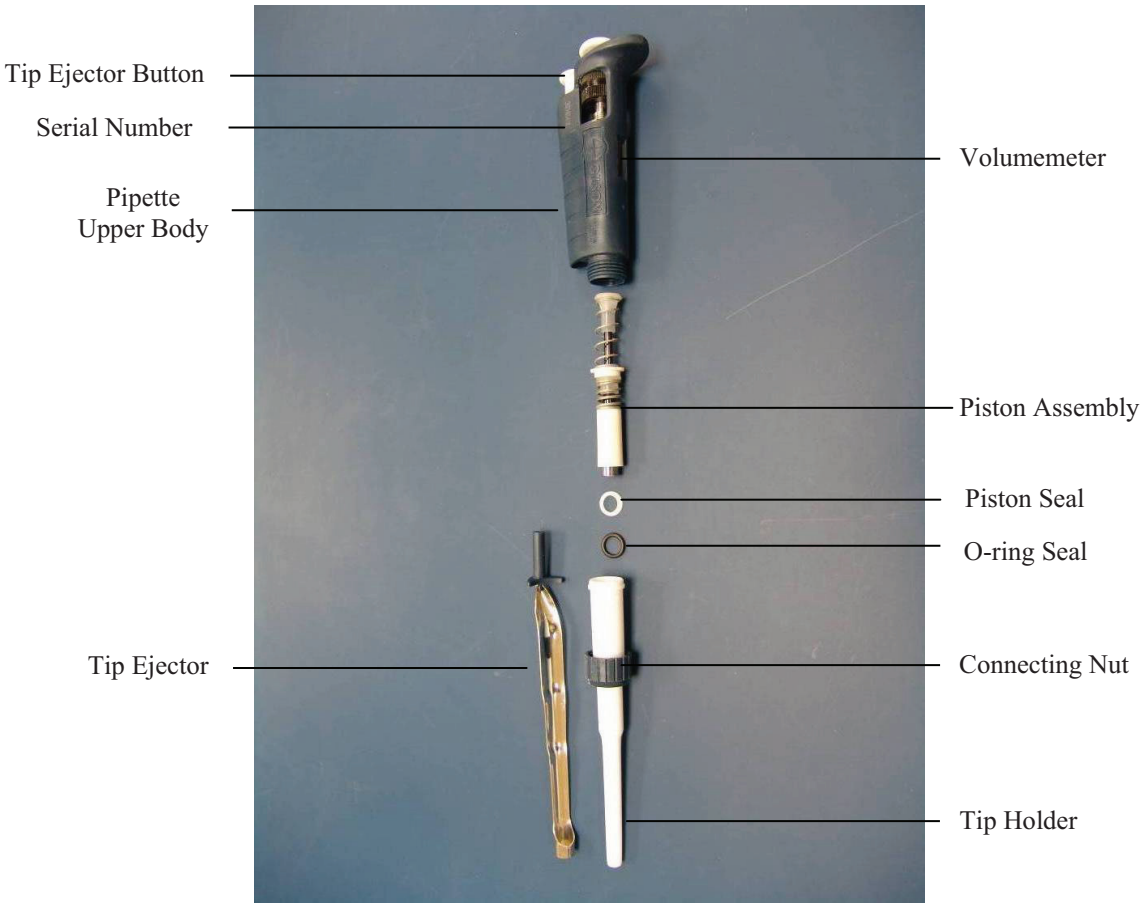

Figure 2: Pipetman® Component Breakout

Immersion Depth and Wait Time		
Model	**Immersion depth (mm)**	**Wait time (sec)**
P2	1mm	1
P10	1mm	1
P20	2mm to 3mm	1
P100	2mm to 4mm	1
P200	2mm to 4mm	1
P1000	2mm to 4mm	2 to 3

Table 1: Immersion chart

Title: Gilson Pipetman® P-20 to P-1000 Operation and Maintenance

Pipeteman Trouble Shooting		
Symptom	**Possible Cause**	**Action**
Pipette is leaking sample	O-ring or seal is worn	Replace both parts.
Pipette will not aspirate	O-ring or seal is worn Tip holder is loose Piston is damaged (chemically or mechanically) Damaged tip holder Connecting nut is loose	Replace both parts. Tighten connecting nut. Return pipette to supplier. Replace the tip holder. Tighten connecting nut.
Pipette is inaccurate	Improper assembly Unscrew tip holder Connecting nut is loose	See "Maintenance" Tighten connecting nut. Tighten connecting nut.
Pipette is not precise	Tip holder is loose Incorrect operator technique Worn O-ring or seal Piston is damaged (chemically or mechanically) Damaged tip holder	Tighten connecting nut. Operator training. Replace both parts. Tighten connecting nut. Return pipette to supplier. Replace tip holder.
Tips fall off or do not fit	Low quality tips Damaged tip holder Damaged tip ejector	Use better quality tips. Replace he tip holder. Replace the tip ejector.
Bent operating rod	Pipette damaged mechanical shock	Return pipette to supplier.
Operating rod has chemical damage	Chemically damaged rod	Return pipette to supplier.
Volumeter digits unclear	Pipette damaged	Return pipette to supplier.
Cannot set the maximum range	Pipette damaged, mechanical shock	Return pipette to supplier.
Volumemeter thumbwheel is hitching	Pipette damaged, mechanical shock	Return pipette to supplier.
Tip ejector bent	Pipette damaged, mechanical shock	Replace ejector.
Tip ejector chemical damage	Chemical damage, damaged ejector	Replace ejector.

Table 2: Troubleshooting guide

r

Title: Gilson Pipetman® Performance Verification SOP

Approvals:
Preparer:___Judith Fitzpatrick_____ Date __13Oct09_____
Reviewer:__Kari Britt_____ Date __13Oct09_____

1. **Purpose:** To verify the calibration of a single channel pipette.
2. **Scope:** Covers the cleaning, decontamination and verification of a single channel pipette.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. balance operation SOP
   4.2. balance calibration SOP
   4.3. autoclave SOP
   4.4. Gilson Pipetman® Operation and Maintenance SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. balance
   7.2. 20g and 200g standard mass weights
   7.3. weigh boats
   7.4. deionized (DI) water
   7.5. small beaker for holding DI water
   7.6. verification labels
   7.7. verification form
   7.8. verification Pass/Fail form
   7.9. pipette tips
   7.10. Pipetman® (P20, P200, and P1000)
   7.11. 70% isopropyl alcohol (IPA)
   7.12. lab towels
   7.13. tweezers
   7.14. thermometer
   7.15. calculator
   7.16. barometer
8. **Procedure:**
   8.1. **Clean the pipette** (See Figure 2.)
   Note: Most pipettes are designed so that the parts that normally come into contact with liquid contaminants can easily be cleaned and decontaminated.
      8.1.1. Wipe entire pipette with a lab towel dampened with a mild detergent solution.
      8.1.2. Wipe entire pipette with a lab towel dampened with distilled water.
      8.1.3. Remove the tip ejector.
      8.1.4. Wipe the tip ejector with a lab towel dampened with a mild soap solution.

Title: Gilson Pipetman® Performance Verification SOP

8.1.5. Wipe the tip ejector with a lab towel dampened with distilled water.
8.1.6. Refit the tip ejector and allow the pipette to dry.
8.1.7. Dispose of lab towels in bio-hazardous waste receptacle.

8.2. **Chemical decontamination**

8.2.1. Spray a lab towel with 70% IPA to dampen the lab towel.
8.2.2. Wipe upper part of body with dampened lab towel.
8.2.3. Wipe tip holder tip ejector with dampened lab towel.
8.2.4. Wipe entire pipette with a lab towel dampened with distilled water.
8.2.5. Leave pipette to dry or wipe pipette dry with lab towel.
8.2.6. Dispose lab towels in bio-hazardous waste receptacle.

8.3. **Verification of Calibration** (See Figure 1 page 12)

Note: To test the accuracy of the pipette you will pipette a set volume 10 times and then weigh the total pipetted volume. 1mL of DI water should weigh 1g and 1µL should weigh 1mg. Calculate your % Error using the equation below:

$$\frac{\text{Expected Mass - Actual Mass}}{\text{Expected Mass}} \times 100 = \% \text{ Error}$$

If the % Error is ≤ 2% the pipette passes verification if it is greater than 2% the pipette fails. We will verify the pipette once at the maximum volume for the pipette, once at the ½ maximum volume, and once at the minimum volume. Altogether you will pipette 30 volumes and weigh 3 times for each pipette.

8.3.1. Record the necessary information on the Verification form. Enter information in the empty box to the right of the box specifying the information.
8.3.2. Verify that the balance is still in calibration with a 20gm and 200g mass.
8.3.3. Verify that the calibration label of the balance is within the dated calibration time period.
8.3.4. Fill a small beaker with DI water.
8.3.5. Place the weigh boat on the balance.
8.3.6. Tare the balance and verify that 0.00 is being displayed.
8.3.7. Verify that the pipette is set to the maximum volume (e.g. the maximum volume for a P-20 pipette is 20µL.).
8.3.8. On the Pipette Verification Form, beside Selected Volume, enter the volume you will be pipetting and the value of that volume times 10 (e.g. for a 20µL pipette you will record 20µL for the selected volume and 200µL for the selected volume times 10.).
8.3.9. Calculate the expected mass by converting the selected volume times 10 using the following conversions: 1µL = 1mg and 1mL = 1g. Use the selected volume times 10 as the volume (e.g. for a 20µL pipette, 200µL multiplied by 1mg/µL = 200mg). Record the expected mass in the box beside Expected Mass.
8.3.10. Verify that the pipette is set to the maximum volume recommended by the manufacturer for the pipette.
8.3.11. Place pipette tip securely on the pipette.

Title: Gilson Pipetman® Performance Verification SOP

8.3.12. Aspirate DI water into pipette tip from the beaker and dispense it into weigh boat. Refer to Figure 1 page 6 for proper operation of the pipette.
8.3.13. Repeat the above step 9 times. Each time you dispense the selected volume mark the Verification form in the numbered box beside Dispense Repetitions.
8.3.14. Record the final mass on the Verification form next to Recorded Mass.
8.3.15. Tare the balance and verify that 0.00 is being displayed.
8.3.16. Set the volume of the pipette to half capacity (e.g. For a P-20 pipette, set it to 10µL.) and verify the volume.
8.3.17. Repeat steps 8.3.9. through 8.3.16 with the pipette set to the half-capacity volume.
8.3.18. Tare the balance and verify that 0.00 is being displayed.
8.3.19. Set the volume of the pipette to the minimum capacity recommended by the manufacturer (e.g. For a P-20 pipette, set it to 2µL.)
8.3.20. Repeat steps 8.3.9. through 8.3.16 with the pipette set to the minimum-capacity volume.
8.3.21. Calculate the % Error (as directed in the note at the beginning of section 8.3) for each test (maximum, half-capacity, and minimum volumes) and record the results on the verification form.
8.3.22. Verify that all fields of the verification form have been filled out and fill out the Pipette Verification Pass/Fail form according to the results of the tests.

9. Attachments:

9.1. Figure 1: Pipetman® Verification Setup
9.2. Figure 2: Pipetman®
9.3. Figure 3: Pipetman® Component Breakout

10. History:

Name	Date	Amendment
Bob O'Brien	10Jul07	Initial Release
Bob O'Brien	11May08	Added photographs and clarified directions. College name change
Judith Fitzpatrick Kari Britt	13Oct09	Added directions for using 3 different volumes for calibrating the same pipette. Title changed from Calibration to Performance Verification

Title: Gilson Pipetman® Performance Verification SOP

Figure 1: Pipetman® Verification Setup

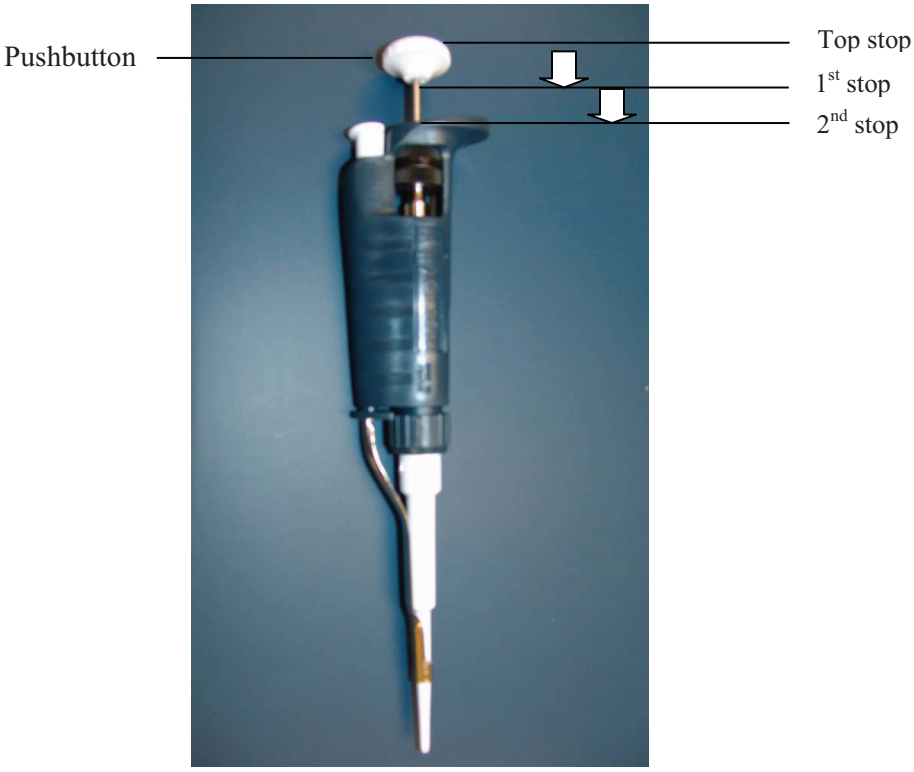

Figure 2: Pipetman®

Title: Gilson Pipetman® Performance Verification SOP

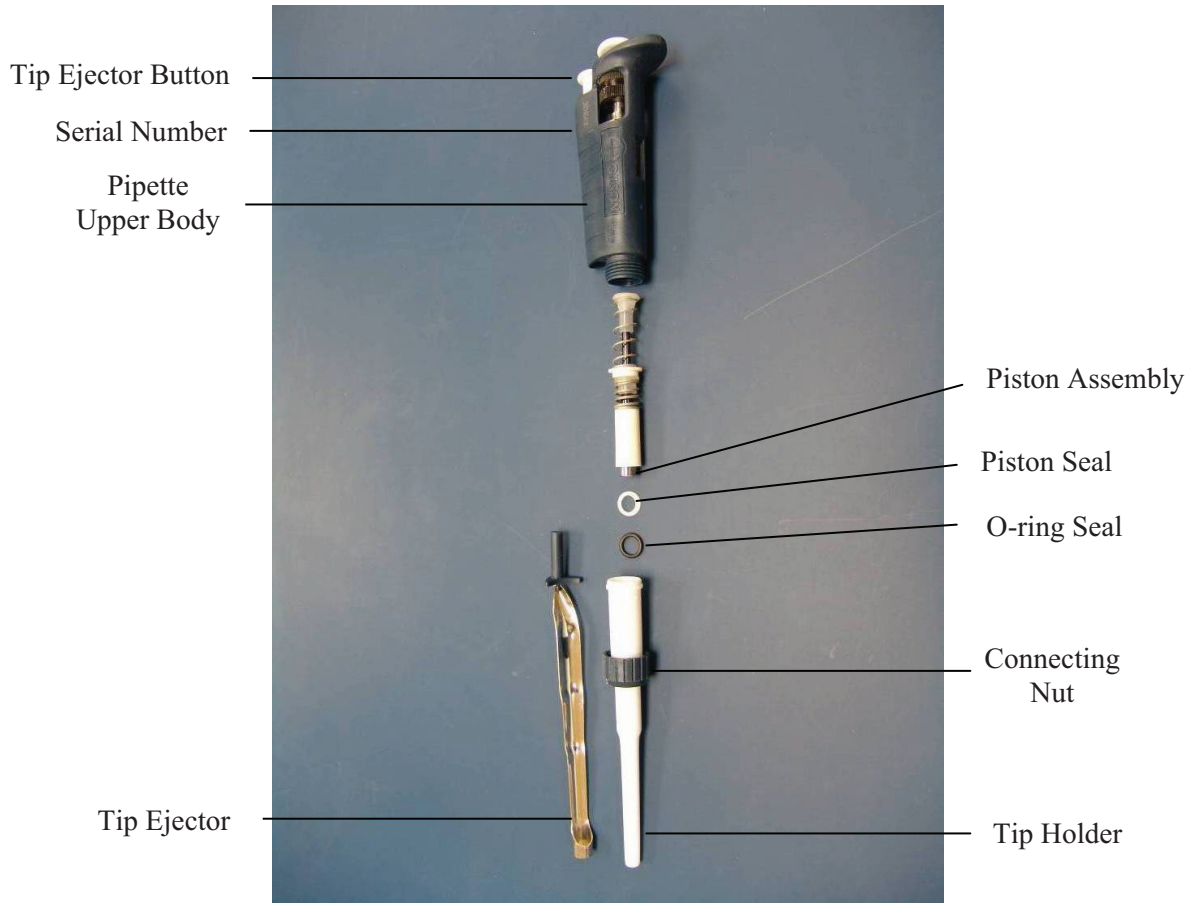

Figure 3: Pipetman® Component Breakout

Great Bay Community College
320 Corporate Drive
Portsmouth, NH 03801

Pipette Performance Verification Form

Pipette Information

Name and Description:_____

Model:_____

Serial Number:_____

Verification		**Pipette**	
Technician		Volume Range	
Date		Number of Channels	

Test Conditions			
Balance Serial #		Balance Model	
Sensitivity		Balance Calibration Date	
Correction Factor		Balance Calibration Technician	
Air Temperature			
Barometric Temperature			
Relative Humidity			

Tests												
Test 1 (Max. volume)												
Selected Volume											Expected Mass	
Selected Volume X 10											Recorded Mass	
Dispense Repetitions	1	2	3	4	5	6	7	8	9	10		
Test 2 (Half cap. volume)												
Selected Volume											Expected Mass	
Selected Volume X 10											Recorded Mass	
Dispense Repetitions	1	2	3	4	5	6	7	8	9	10		
Test 3 (Min. volume)												
Selected Volume											Expected Mass	
Selected Volume X 10											Recorded Mass	
Dispense Repetitions	1	2	3	4	5	6	7	8	9	10		

Test results	
% Error Test 1	
% Error Test 2	
% Error Test 3	
Pass or Fail	

Pipette Performance Verification Pass/Fail Form

Pipette Information

Name and Description: _____

Model: _____

Serial Number:_____

Verification Comments:

_____

_____

_____

Technician:_____

Date:_____

Passed Verification:	**Failed Verification:**
Date of Verification_____ Technician_____ Verification Sticker: □Yes □ No □ Not Applicable Next Verification Due Date_____	Reason for Failure_____ _____ _____ Date Out of Service_____ Technician_____

Name	Date	Amendment
Bob O'Brien	08Jun06	Initial release
Bob O'Brien	02Jun08	College name change
Mary Jane Kurtz and Sonia Wallman	23Nov09	Changed form name from Calibration Form to Pipette Performance Verification Pass/Fail Form

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_____________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Calibration
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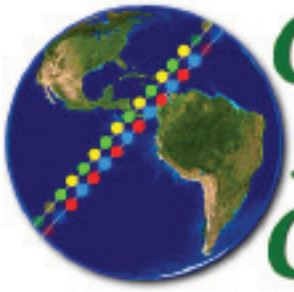
Maintenance/Instrumentation Technician

REF	Key Functions & Tasks (Maintenance/Instrumentation Technician)
1	**Work in compliance with EH&S.**
1.a	Wear appropriate personal protective equipment
1.b	Participate in emergency drills and emergency response teams
1.c	Identify unsafe conditions and take corrective action
1.d	Appropriately and safely access production equipment
1.e	Handle, label, and dispose of hazardous / biohazard materials
1.f	Access and utilize MSDS
1.g	Comply with all permitting requirements (LOTO, hot work, line breaking, CSE)
1.h	Carries out operations with attention to OSHA and EPA regulations, and any other applicable state and federal regulations
1.i	Carries out operations with attention to OSHA and EPA regulations, and other applicable state and federal regulations.
1.j	Participate in all company safety training and audits as required
1.k	Assists with waste treatment operations
2	**Work in compliance with cGMPs.**
2.a	Follow SOPs for all operations
2.b	Prepare required documentation for recording and notification of events and changes related to equipment, including: as-found/as-left data, maintenance logs, calibration certificates and instrument labels, deviations, OOT reports, and installation reports
2.c	Maintain equipment logbooks
2.d	Control and receive parts and materials.
2.e	Maintain training documentation
2.f	Maintain equipment and process utilities in a validated state.
2.g	Follow appropriate gowning procedures and behaviors for work in controlled/classified areas.
2.h	Ensure appropriate flow of personnel, equipment, and materials
2.i	Follow change control procedures for process, equipment, and documentation.
2.j	Update status of equipment and materials
2.k	Identify and report exception events and CAPA.
3	**Monitor, maintain, and repair process or laboratory equipment.**
3.a	Monitor, maintain and repair process vessels and fluid handling systems including pumps, pipes and valves.
3.b	Monitor, maintain and repair heat transfer equipment (heat exchangers, condensers, evaporators).
3.c	Monitor, maintain and repair process support equipment (autoclaves, ovens, dryers, washers).
3.d	Monitor, maintain and repair refrigeration equipment (freezers, lyophilizers).
3.e	Monitor, maintain and repair laboratory equipment (scales, hot plates, HPLC).
3.f	Monitor, maintain and repair fill/finish equipment including automated equipment (filling machines, cap/inspect machines, packaging).
3.g	Respond to alarms per procedure.
4	**Monitor, maintain, and repair plant utility systems.**
4.a	Monitor, maintain and repair utility systems including instrument air, process gases (CDA, nitrogen, argon, CO2).
4.b	Monitor, maintain and repair utility systems including steam, process water types (DI, WFI, USP) and cooling/heating fluids (glycol, liquid nitrogen).
4.c	Monitor, maintain and repair utility systems including HVAC, electrical, hydraulic.
4.d	Monitor, maintain and repair waste treatment systems (waste neutralization, inactivation, scrubbers).
4.e	Monitor, maintain and repair site safety systems (fire suppression, eye wash, safety showers, etc.)
4.f	Monitor, maintain and repair security system including controlled personnel access.
4.g	Monitor building management systems (BAS).
4.h	Respond to alarms per procedure.
4.l	Perform testing of plant utilities.

REF	Key Functions & Tasks (Maintenance/Instrumentation Technician)
5	**Calibrate Control System Components.**
5.a	Calibrate pressure, temperature, flow, weight, pH, DO, and other critical measurement devices and transmitters.
5.b	Calibrate control valves, actuators, positioners, and process switching devices.
5.c	Investigate, correct and document calibration failures (identify OOS results and report in CAPA).
5.d	Use calibration reference standards and other specialized equipment.
6	**Maintain control systems, equipment and instrumentation.**
6.a	Program and/or replace microprocessors, PC, PLC, and DCS controllers and devices.
6.b	Maintain and repair on-off actuators, diaphragms, electrical and pneumatic and hydraulic industrial control circuits and equipment.
7	**General Engineering Technology and Maintenance Skills**
7.a	Solve system/equipment problems using P&IDs, manuals, and other drawings as needed.
7.b	Participate in root cause analysis and verification, identification of solutions, and development of solution implementation work plans
7.c	Assist in installation, modification, commissioning, and validation of equipment.
7.d	Communicate with all applicable departments about scheduling, service needs and priorities, problems, status of equipment and repair jobs, and status of utility systems.
7.e	Maintain spare parts inventory.
7.f	Communicate with vendors and service suppliers to coordinate and schedule external calibration and maintenance activities.
7.g	Use Computerized Maintenance Management System (CMMS) to schedule and track work orders for corrective and preventive maintenance (PMs) (Maximo, Atlas, Blue Mountain).

Validation



GLOBAL
BIOMANUFACTURING
CURRICULUM

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Example of an Autoclave IQ protocol:

AUTOCLAVE IQ

Objective

To demonstrate that the Autoclave manufactured by ____, model # _____ and accessories installed in building ____, room ___ conforms to the purchase specifications and the manufacturers literature, and to document the information that the equipment meets specifications.

Scope

For new installation, modification, replacement, or relocation of any critical component of the autoclave.

Responsibility

Supervisor of the Department where the autoclave is located is responsible for writing the protocol, supervising the performance of the IQ, verifying the data and writing the IQ report.

QA is responsible for approving the protocol and reviewing and approving the data and conclusions.

Systems/Equipment

Give a brief description of the autoclave indicating the manufacturer and model name/number, where it is located, what materials it will be sterilizing, any accessories that accompany it (e.g. carts) and provide a short description of how the autoclave functions.

Component List

Typical major components associated with autoclaves are:

> autoclave chamber, baffles, shell insulation, frame, doors, door seals, temperature detectors and probes (RTDs), temperature recording chart, safety valves, vacuum pump, side door motor, sterilization cart, pressure transmitters and gauges, microcomputer control, chamber high water sensor .

Procedure

Fill in the prepared checklists with the detailed mechanical and electrical specifications, drawings, etc. (as itemized in the IQ format) for each component as listed in the IQ format.

The individual component checklist includes a space to record the information plus any deviations found during the installation check.

Reporting

Responsible person verifies that the information is complete, prepares the Deviation Report and the Installation Qualification Report and, submits to QA for review and approval.

Example of an OQ protocol for an autoclave:

AUTOCLAVE OQ

Objective

To determine that the autoclave model # _____ , installed in building ___, room ___ operates according to specifications, to determine the heat /steam distribution in the jacket and empty chamber and to record all relevant information and data to demonstrate it functions as expected.

Scope

a) For new installation, modification, replacement, or relocation of any critical component of the autoclave.

b) If there is a contamination problem.

To be performed after the IQ has been completed and approved.

Responsibility

Supervisor of the Department where the autoclave is located is responsible for writing the protocol, supervising the performance of the OQ, verifying the data and writing the OQ report.

QA is responsible for approving the protocol and reviewing and approving the data and conclusions.

Equipment and Documents

Example of calibration instruments required are:

thermocouples, pressure calibrator, vacuum calibrator, temperature detectors and probes, timers, temperature bath, flow meters. (Certification methods should be referenced)

SOP# ___: Operation, Maintenance, and Calibration of the Autoclave

Training records for personnel operating and maintaining the autoclave.

The calibrating instruments must be certified before being used for calibrating the autoclave.

Procedure:

Typical critical parts of the autoclave to be calibrated are:

temperature sensors, pressure sensors, pressure gauges, pressure switches, pressure transmitters and input/output transmitter.

Typical alarm points to be checked on the autoclave are:

under or over temperature, evacuation too long, sterilization too long, vacuum system failure, door open, failure reading temperature or pressure or both, failure reading load, pressure in chamber with door unsealed, chamber flooded, insufficient vacuum level to perform leak test, low battery,

Proceed with the testing of the functions of the autoclave.

Title: Operational Qualification Protocol for Autoclave

Approvals:
Preparer:_____Kari Britt_____ Date _____10Aug10_____
Reviewer:_____Sonia Wallman_____ Date_____ 10Aug10_____

1. **Purpose:**
   1.1. Proper use of BTSure biological indicators to carry out an operational qualification of an autoclave.
2. **Scope:**
   2.1. To validate the ability of a laboratory autoclave to sterilize.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. BTSure Biological Indicator manufacturer instructions
   4.2. autoclave SOP
   4.3. incubator or waterbath SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. The contents of the BTSure biological indicator is extremely hot and under pressure after autoclaving. It may burst and cause burns. Allow at least 10 minutes to cool before handling it.
   6.2. Always wear the appropriate personnel protective equipment.
7. **Materials:**
   7.1. BTSure biological indicator unit(s)
   7.2. BTSure crusher
   7.3. autoclave
   7.4. incubator (55-60ºC) or waterbath
   7.5. small beaker
   7.6. aluminum Foil
   7.7. permanent marker
8. **Procedure:**
   8.1. Remove an appropriate number of BTSure units from the box.
   8.2. Remove one unit for each area of the autoclave to be tested and one additional unit to be used as a positive control (this one will not be placed in the autoclave).
   8.3. Label indicators with appropriate information.
   8.4. Place each unit in a small beaker so that they lay horizontally in the bottom of the beaker. Use a separate beaker for each BTSure unit.
   8.5. Cover the beakers with aluminum foil.
   8.6. Place the beakers inside the autoclave except the positive control.
      8.6.1. One of the beakers should be situated so that it is directly over or next to the drain.

Title: Operational Qualification Protocol for Autoclave

Note: The area surrounding the drain is the coolest part of the autoclave and considered to be the least effective area for sterilization.

8.7. Operate the autoclave per autoclave SOP.

8.8. Remove the beakers from the autoclave. Allow indicators to cool for at least 10 minutes.

8.9. Remove the biological indicators from the beakers.

8.10. Observe the color change (blue to black) of the chemical indicator on the BTSure label.
Note: Color change indicates exposure to steam. It does not indicate acceptable sterilization.

8.11. **Incubation**

8.11.1. Place an indicator into the crusher in an upright position and squeeze the crusher to break the glass ampoule. This will allow the strip to be immersed in the media.

8.11.2. Repeat the crushing with each additional indicator including the positive control.

8.11.3. Immediately place the indicators (including the positive control) into the incubator or waterbath.

8.11.4. Incubate at 55 to 60°C for at least 48 hours.

8.12. **Interpretation**

8.12.1. During the incubation examine the indicators at regular intervals starting approximately at 4 hours, and again at approximately 8, 12, 16, 24 and 48 hours for any change in color. Record results on the Autoclave Monitoring Form.

8.12.1.1. The positive control tube should change from purple media to yellow. If it does not, the study is invalid and needs to be repeated with a new lot of indicators.

8.12.1.2. The sample indicators should not change color. No color change (media remains purple) indicates adequate sterilization.

8.12.1.3. If the sample indicators turn yellow this indicates bacterial growth and an investigation will need to be conducted. This may include re-performing the autoclave cycle.

8.12.2. Report any indication of bacterial growth to instructor or lab manager.

8.12.3. Dispose all used BTSure tubes in a biohazard receptacle when finished.

9. Attachments:

9.1. Figure 1: Biological Indicator in Plastic Crusher

10. History:

Name	Date	Amendment
Hope Townes and Kari Britt	25Jan06	Initial release
Bob O'Brien	20Jul06	Added photograph and disposal information.
Bob O'Brien	20Aug06	Added interval hours for examination.
Bob O'Brien	12Feb07	Updated date format.
Bob O'Brien	11Apr08	College name change
Sonia Wallman	15Oct09	Additional information to purpose and scope.
Kari Britt	10Aug10	Added data table. Made formatting edits throughout.

Title: Operational Qualification Protocol for Autoclave

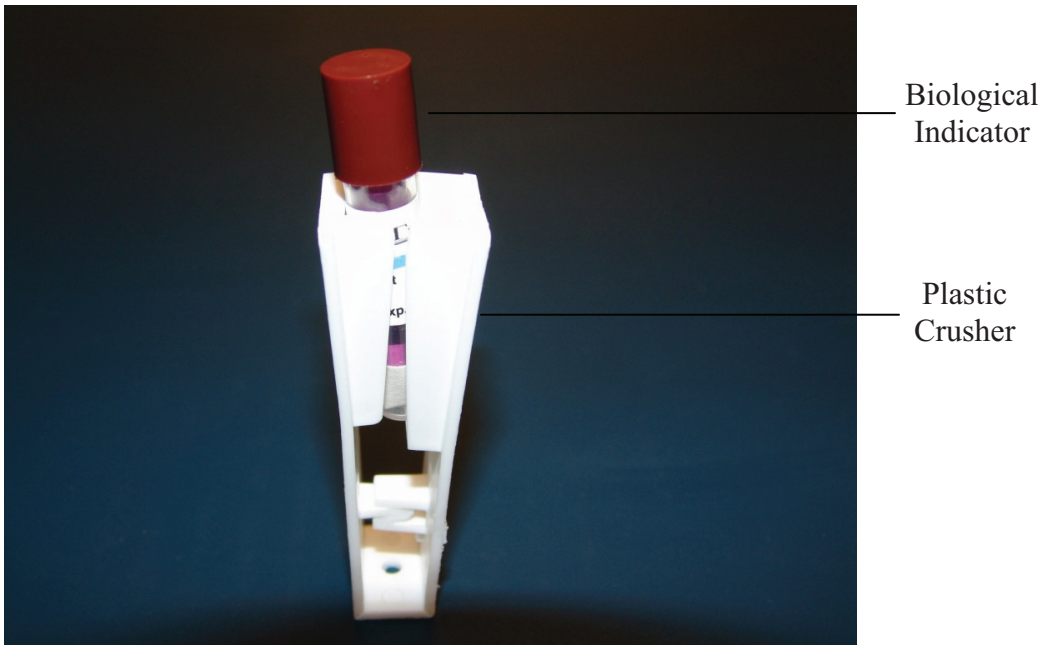


Figure 1: BTSure Biological Indicator in Plastic Crusher

Title: Market Forge Sterilimatic Autoclave SOP

Approvals:
Preparer: ____ Bob O'Brien _____ Date _____03Apr08_____
Reviewer: ___ Deb Audino _____ Date _____03Apr08_____

1. **Purpose:**
   1.1. To learn the proper operation of the Market Forge Autoclave.
2. **Scope:**
   2.1. To properly sterilize equipment, solutions and biohazardous waste.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Market Forge Manufacturers instructions.
   4.2. Market Forge Autoclave Cleaning SOP.
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Open autoclave door while standing at arm lengths away; do not put face near door. Steam from autoclave can cause serious burns.
7. **Materials:**
   7.1. Market Forge's "Sterilmatic" Sterilizer, Model STM-E, Type C
   7.2. gallon jug of deionized (DI) water
   7.3. items to be sterilized
   7.4. autoclave indicator tape
   7.5. protective autoclave gloves and safety glasses
   7.6. drain Bucket
8. **Procedure:**
   8.1. Close drain. Do not over-tighten or the autoclave will leak.
   8.2. Fill autoclave with about 1 gallon of deionized water. Level should be just below the lower edge of the autoclave door.
   8.3. Make sure all items to be autoclaved are labeled appropriately (i.e. contents/prep date initials).
   8.4. Place a small piece of autoclave indicator tape on each item.
   Note: It is best to autoclave dirty and clean media separately. It is also better to autoclave liquid and non-liquid items separately.
   8.5. Make sure caps on all containers are very loose. Flasks of media should be plugged with gauze or loosely covered with a cap or aluminum foil.
   8.6. Place items into autoclave. Tall items fit best in the back.
   8.7. Close and lock the door. Push the handle down as far as it will go after it is latched.
   8.8. Set exhaust to SLOW if liquids are being autoclaved, otherwise, set to FAST. The slow setting will prevent liquids from boiling over.

Title: Market Forge Sterilimatic Autoclave SOP

8.9. Check that the main power is on. The large switches on the wall to the right side of the autoclave are the power switches.
8.10. Set the timer.
8.10.1. Set to 20 minutes unless another time is indicated by a process SOP.
8.11. Setting the timer starts the autoclave. However, the timer will not move until the temperature is at least 121°C.
8.12. Verify that the temperature reaches at least approximately121°C and that the pressure is approximately 15psi. Record the temperature and pressure on the log sheet.
Note: It takes approximately 10 minutes for the autoclave to cycle-up to operating temperature and pressure. After it reaches the operating levels, the indicator light comes on.
Note: The autoclave is noisy during normal operation.
8.13. After the autoclave cycle, wait until the pressure is below 5psi and the temperature is less than 80 ºC, put on safety glasses and protective gloves, then crack the door to allow steam to escape.
8.14. Let the items in the autoclave cool so as to avoid handling superheated liquids. Take out items using the protective gloves. Be careful not to compromise sterility.
8.15. When cool, drain the water from the autoclave into a container and discard in sink.
8.16. Leave autoclave door and drain open to air dry.
8.17. Wash as needed to remove any spills according to the autoclave cleaning SOP.

9. Attachments:

9.1. Market Forge Autoclave

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial release
Sue Penney	2004	Updated directions.
Jenifer Hall	25Jan05	Put into 2005 SOP formats
Ed Ribitzki	23Sep05	Added note about 10 minute cycle-up time. Added autoclave cleaning SOP. Enhanced photos and added lines pointing to gauges.
Bob O'Brien	08Feb07	Updated date and history format.
Bob O'Brien	04Apr08	College name change

Title: Market Forge Sterilimatic Autoclave SOP

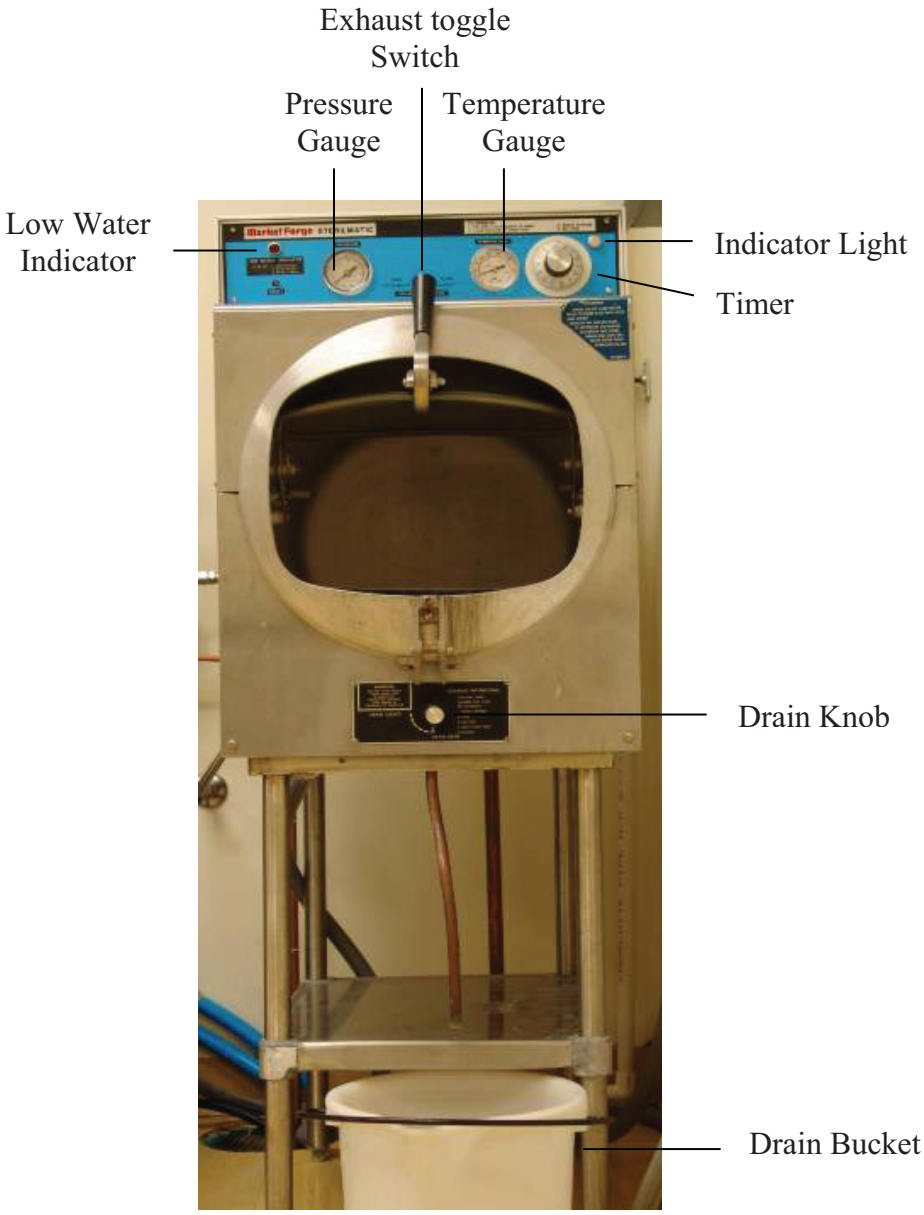


Figure 1: Market Forge Autoclave

Great Bay Community College
320 Corporate Drive
Portsmouth, NH 03801

Autoclave Monitoring Form

Autoclave Information

Name and Description:_____

Model:_____

Serial Number:_____

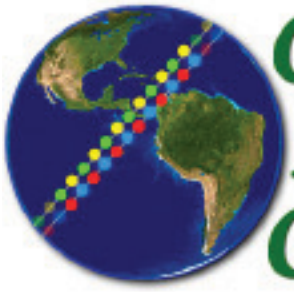
Biological Indicator Test Results

Date	Time	Hours since incubation began	Color of media inside indicator	Comments	Initials

Monitoring Results:	**Failed Monitoring:**
Dates Monitoring Protocol Performed: _____ Monitoring Protocol Initiator: _____ Pass: □Yes □ No	Reason for Failure:_____ _____ _____ Date Out of Service:_____ Technician:_____

Validation Competencies List	QC CHEM	QC MICRO	MANUF UP	MANUF DOWN	MAINT
General Industry Knowledge					
Industry Overview					
Understand the biomanufacturing and pharmaceutical industry, including understanding of the typical product development and manufacturing process, as well as the regulatory environment.	x	x	x	x	x
Appreciate the design and validation of biomanufacturing and/or other types of pharmaceutical manufacturing facilities.			x	x	x
Understand common materials of facility constructionKnowledge of principles of adjacency and flow in pharmaceutical operations			x	x	x
Understand and apply principles of adjacency, flow, and product segregation in pharmaceutical operations.			x	x	x
Validation					
Understand principles of validation, commissioning, and qualification.	x	x	x	x	x
Execute IQ/OQ/PQ protocols for facilities and/or process equipment and/or laboratory equipment	x	x	x	x	x
Understand the principles involved in choosing key process parameters to validate and setting operational specifications.	x	x			
Understand the principles involved in validating new analytical methods					x

EH&S



GLOBAL
BIOMANUFACTURING
CURRICULUM

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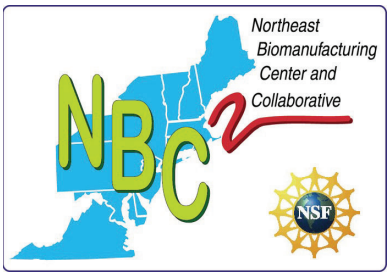


Northeast Biomanufacturing Center and Collaborative

Hazard Communication Program (HCP)

NBC-HCP-000

Date Issued: **06/27/06**
Date Revised: **05/30/07**

APPLICIPABLE STANDARD: 29CFR1910.1200
OSHA's Hazard Communication Standard

Document Contents

SECTION CONTENT
A Application
B Purpose
C Definitions
D Scope
E Chemical Inventory List
F MSDS
G Labeling
H Training
I Outside Contractors

Section A

Application

The Northeast Biomanufacturing Center and Collaborative (NBC^2), under OSHA's Hazard Communication Standard (HCS), requires chemical manufacturers or importers to assess the hazards of all chemicals they produce or import and transmit that information to affected employers and employees. The standard requires the Center to provide employee information about the potential of hazardous chemical exposure under normal use conditions or in a foreseeable emergency; and the transmittal of this information by means of a comprehensive Hazard Communication Program which includes container labeling and other forms of warning, Material Safety Data Sheets, and employee training.

In order to comply with the Hazard Communication Standard, this Hazard Communication Program has been established for NBC^2. All schools, colleges, and administrative offices operating within the center are incorporated within this program.

Primary responsibility for compliance with the Hazard Communication Program lies with the individual institution, laboratory, or facility operating within the center. Each dean, director, chair, and lab supervisor is responsible for safety performance and hazard communication within their respective institutions and labs. The Environmental Health and Safety Department

(EH&S) is responsible development and coordination of policies and procedures. EH&S also provides technical assistance in establishing procedures and monitoring performance in activities involving the Hazard Communication Program.

NBC^2 is committed to creating, maintaining and promoting a safe and healthful environment for all associated individuals including students, faculty, staff employees, hospital patients, and visitors. A critical component of NBC^2's environmental health and safety commitment is integrating information concerning chemical hazards into all academic and operational activities by means of this Hazard Communication Program.

This program outlines the definitions, procedures and training requirements to be utilized by NBC^2 employees and trainees to understand and comply with the Hazard Communication Standard. It is the duty of each employee to become familiar with the contents of this program and ensure compliance with its procedures. Supervisors and instructors shall ensure that employees and trainees understand the details of this program and ensure that employees receive the proper training. Supervisors and instructors are also responsible for maintaining records of this training. These records must be current and readily available for review.

ASSOCIATED DOCUMENTS

NBC-HCP-001: Safety self-audit
NBC-HCP-002: Guidance Document

Section B

PURPOSE

- The NBC2 Hazard Communication Program (HCP) was developed to:
- Inform employees of the hazards associated with chemicals in the workplace.
- Ensure safe use, handling and disposal of hazardous chemicals in the workplace.
- Comply with the Occupational Safety and Health Administration's (OSHA) Hazard Communication Standard (29 CFR 1910.1200)
- A successful Hazard Communication Program will reduce potential incidents of chemical source illnesses and injuries.

Section C

Definitions

Chemical means any element, chemical compound or mixture of elements and or/ compounds.

Chemical manufacturer means an employer with a workplace where chemical(s) are produced for use or distribution.

Chemical name means the scientific designation of a chemical in accordance with the nomenclature system developed by the International Union of Pure and Applied Chemistry (IUPAC) or the Chemical Abstracts Service (CAS) rules nomenclature, or a name, which clearly identify the chemical for the purpose of conducting a hazard evaluation.

Combustible liquid means any liquid having a flashpoint at or above 100 degree F, but below 200 degree F, except any mixture having components with flashpoints of 200 degree F, or higher, the total volume of which make up 99% or more of the total volume of the mixture.

Common name means any designation or identification such as code name, code number, trade name, brand name or generic name used to identify a chemical other than by its chemical name.

Container means any bag, barrel, bottle, box, can, cylinder, drum, reaction vessel, storage tank, or the like that contains a hazardous chemical.

Distributor means a business, other than a chemical manufacturer or importer, which supplies hazardous chemicals to other distributors or to employers.

Employee means a worker who may be exposed to hazardous chemicals under normal operating conditions or in foreseeable emergencies.

Employer means a person engaged in a business where chemicals are either used, distributed, or are produced for use or distribution, including a contractor or subcontractor.

Explosive means a chemical that causes a sudden, almost instantaneous release of pressure, gas, and heat when subjected to sudden shock, pressure, or high temperature.

Exposure or exposed means that an employee is subjected in the coarse of employment to a chemical that is a physical or health hazard, and includes potential exposure.

Flammable means a chemical that falls into one of the flowing categories:

- "Aerosol, flammable" means an aerosol that, when tested by the method described in 16 CFR 1500.45 yields a flame projection exceeding 18 inches at full valve opening, or a flashback at any degree of valve opening
- "Flammable Gas" means a gas that, at ambient temperature and pressure, forms a flammable mixture with air at a concentration of thirteen percent by volume or less; or a gas that at ambient temperature and pressure, forms a range of flammable mixtures with air wider than twelve percent by volume, regardless of the lower limit
- "Flammable Liquid" means any liquid having a flashpoint below 100 degree F, except any mixture having components with flashpoints of 100 degree F or higher, the total of which make up 99% or more of the total volume of the mixture
- "Flammable Solid" means a solid, other than a blasting agent or explosive as defined in CFR 1971.109, that is liable to cause fire through friction, absorption of moisture, spontaneous chemical change, or retained heat from manufacturing or processing, or which can be ignited readily and when ignited burns so vigorously and persistently as to create a serious hazard

Flashpoint means the minimum temperature at which a liquid gives off a vapor.

Foreseeable emergency means any potential occurrence such as, but not limited to, equipment failure, rupture of containers, or failure of control equipment, which could result in an uncontrolled release of a hazardous chemical into the workplace.

Hazardous chemical means any chemical, which is a physical hazard or a health hazard.

Hazard warning means any words, pictures, symbols, or combination thereof appearing on a label or other appropriate form of warning which convey the specific physical and health hazard including target organ effects, of the chemicals in the containers.

Health hazard means a chemical for which there is statistically significant evidence based on at least one study conducted in accordance with the established scientific principles that acute or chronic health effects may occur in exposed employees.

Identity means any chemical or common name, which is indicated on the material safety data sheet (MSDS) for the chemical.

Immediate use means that the hazardous chemical will be under the control of and used only by the person who transfers it from a labeled container and only within the work shift it is transferred.

Label means any written, printed, or graphic material displayed on or affixed to containers of hazardous chemicals

Material Safety Data Sheets (MSDS) means written or printed material concerning a hazardous chemical.

Mixture means any combination of two or more chemicals if the combination is not, in whole or in part, the result of a chemical reaction.

Oxidizer means a chemical other than a blasting agent or explosive as defined in CFR 1910.109(a), that initiates or promotes combustion on other materials, thereby causing fire either of itself or through the release of oxygen or other gases.

Physical hazard means a chemical for which there is scientifically valid evidence that it is combustible liquid, compressed gas, explosive, flammable, an organic peroxide, an oxidizer, pyrophoric, unstable or water-reactive.

Produce means to manufacture, process, formulate, blend, extract, generate, emit, or repackage.

Responsible party means someone who can provide additional information on the hazardous chemical and appropriate emergency procedures, if necessary.

Specific chemical identity means the chemical name, Chemical Abstracts Service (CAS) Registry Number, or any other information that reveals the precise chemical designation of the substance.

Unstable (reactive) means a chemical which in the pure state, or as produced or transported, will vigorously polymerize, decompose, condense, or will become self-reactive under conditions of shocks, pressure, or temperature.

Use means to package, handle, react, emit, extract, generate as a byproduct, or transfer**.**

Water-reactive means a chemical that reacts with water to release a gas that is either flammable or presents a health hazard.

Work area means a room or defined space in a workplace where hazardous chemicals are produced or used, and where employees are present.

Workplace means an establishment, job site, or project, at one geographical location containing one or more work areas.

Section D

SCOPE

This program applies to:

- All departments which use or store chemicals and
- All chemicals used by employees under normal conditions of work or in foreseeable emergencies.

EXCEPTION: This program does not apply to research laboratories. These facilities are subject to Chemical Hygiene Plan requirements under the OSHA Laboratory Standard, 29 CFR 1910.1450. For more information, contact your Chemical Hygiene Officer.

Section E

CHEMICAL INVENTORY LIST

In Appendix A of this program is a blank Chemical Inventory List Form. All chemicals being used in this department and for which there is an MSDS in the binder are to be listed on this Inventory form. The completed, current Inventory is to reside in the MSDS binder kept in this department.

Procedure for maintaining the inventory list:

1. When a chemical product is received at this department, retrieve the Program binder.

2. Check the Inventory List for the name of the chemical product as it appears on the MSDS accompanying the shipment.

3. If the product is not listed, add it to the list.

Section F

MSDS

An MSDS must be kept on file for all chemicals on the Inventory list. MSDS's are designed to provide the information needed to handle chemicals safely. MSDS's may differ somewhat in format and content, however all should contain the following:

1. Substance identification - names, synonyms, manufacturer contact information, and index numbers.
2. List of active and inert ingredients - components and contaminants.
3. Exposure limits - ACGIH, TLV, OSHA PEL, etc.
4. Physical data – boiling, melting points, vapor pressure, evaporation rate, specific gravity or density, water solubility, physical description.
5. Fire and explosion data – LEL, flashpoint, flammability, class of hazardous atmosphere, firefighting media and methods, including fire extinguishers, etc.

6. Transportation requirements, if any
7. Toxicity and health hazard data - including target organ, specific acute and chronic health effects, potential cancer risk, first aid and emergency medicine.
8. Storage and disposal - including reporting requirements
9. Spill and emergency response procedures.
10. Measures to protect employees including personal protective equipment, safety shower and eyewash, etc.

Please contact EHS or the Chemical Hygiene Officer if an MSDS appears to be inadequate, illegible, out-of-date or incomplete.

MSDS Procedures

1. Obtain and label one or more three ring binders and label "MSDS's". Place all old and new MSDS's in the binder(s). File MSDS's alphabetically and by use, location or other suitable category (this may already have been done in many departments).
2. Check all deliveries of chemicals for the MSDS(s). An MSDS should accompany the first shipment of all new or re-formulated chemicals.
3. When a chemical is received with an MSDS, place it in the binder and add the product name to the Chemical Inventory List. Discard any old or out-of-date MSDS for the same or similar product that is no longer in stock.
4. If a chemical is received without an MSDS, check the MSDS binder to determine if it already contains the MSDS. If not, immediately request one from the supplier. Store the chemical separately, label "DO NOT USE" and do not use until the MSDS is received.
5. MSDSs can also be obtained from vendor web sites.
6. Inform all employees of the location of the MSDS binder.

MSDS Location_____

Use of MSDS in Exposure Incidents

If an employee is exposed to a chemical and the exposure results in an illness or injury that requires treatment by medical personnel:

1. Ensure that medical personnel see the individual immediately.

2. Provide a copy of the MSDS to the medical personnel involved. Along with the MSDS provide any additional information you have on the chemical and when, where and how it was used.

Section G

LABELING

Primary container label contents - Labels on all primary containers must include:

1. The identity of the chemical - common name &/or chemical name.
2. A hazard warning – such as "Caution, Warning, Flammable, Toxic", etc.
3. The name and address of the manufacturer.
4. Chemical hazard ratings for health, fire reactivity (HMIS)
5. Target organs that may be affected by chronic health hazards

Anytime a container contains the information listed above, an additional HMIS label will not be required. We will accept the manufactures original label if it meets this criteria.

Primary container label procedure:

1. When chemicals are received, check all containers to ensure that the product label meets the requirements outlined above.
2. With each chemical shipment the purchasing agent or his/her designee will check all containers to ensure that the condition is safe and that all labels meet the requirements outlined in this program. Do not accept unsafe containers or improperly labeled containers.

A secondary container is any container other than the one in which the chemical was received from the supplier. Secondary container labels will contain the same information as labels for primary containers. All labeling information can be obtained from the original container, or the MSDS for the product. Label secondary containers if:

- More than one employee uses the container.
- The container is used longer than one shift or left unattended in the work area.
- Labels on containers not containing original product will be removed and re-labeled
- Any portable containers used to store, transport or transfer chemicals which hold a sufficient amount to present a physical or health hazard must be labeled

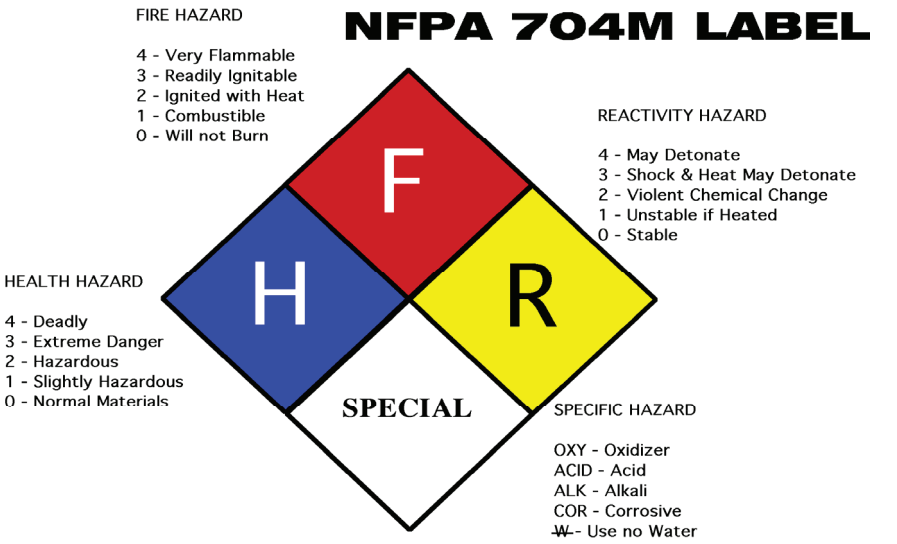
It is not necessary to label the secondary container if:

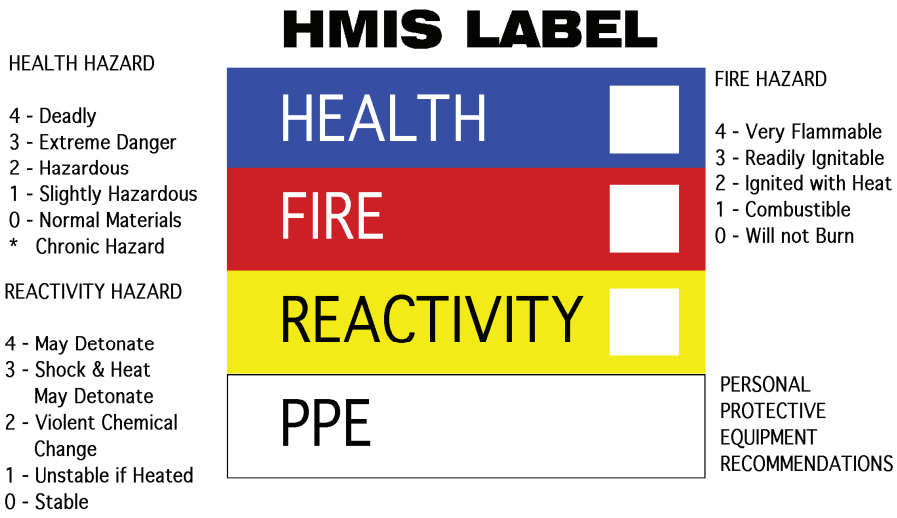
- one employee uses the chemical without exposing others, and
- returns the contents to the original container or disposes of the rest of it.

We will be using the National Fire Protection Association (NFPA) or Hazardous Material Information System (HMIS) to label items that are not correctly labeled or items that do not have labels. This includes numerical ratings for the acute health, flammability and reactivity hazard, the assignments of a personal protective equipment index and the designation of chronic health hazards

The hazard communication portion of the NFPA communicate information on:

1. Chemical identify – common names and code numbers
2. Degree of Acute Health, Flammability and Reactivity hazards – numerical rating
3. Proper Personal Protective Equipment – pictograms
4. Chronic Health Hazards





Section H

TRAINING

Training Matrix - Use this table to determine training requirements. These requirements apply to all employees who will use hazardous chemicals in the course of their job duties. Please contact NBC2 for assistance with your training needs

When	Content	Training methods
Initially, prior to assignment to work	• Details of this program • OSHA requirements • Physical, health hazards of exposure to hazardous chemicals • How to use MSDS's, labels (and other warnings if any) • Location of MSDS's, inventory list and copies of this program. • How to detect presence or release of hazardous chemicals. • Measures to protect employees including safe work practices, PPE, and emergency procedures	Classroom type training. Video, other AV and interactive media are useful for this application
Upon introduction of	• Physical, health hazards of exposure to hazardous chemicals	Safety meeting, job, facility or task orientation

new hazards, (new chemicals, new tasks, etc.)	• Measures to protect employees including safe work practices, PPE, and emergency procedures	
Upon assignment to non-routine tasks	• Physical, health hazards of exposure to hazardous chemicals • Measures to protect employees including safe work practices, PPE, and emergency procedures	Safety meeting should include walkthrough and task orientation.

Keep these training records:

- Date of training.
- Name and job title of trainer.
- Names of the trainees.
- Training topics.
- Other pertinent information to substantiate the training

Note: Please see Training Record Form, Appendix B.

Section I

OUTSIDE CONTRACTORS

During a pre-job walkthrough or meeting:

- Inform contractors of any hazards in the work area that their employees may encounter during the term of the contract.

During the term of the contract, observe work practices to ensure that contractors are complying with OSHA. Contractors are required to observe the following guidelines (these guidelines apply to all sub-contractors also):

- Establish and enforce safe work practices.
- Comply with all applicable OSHA requirements
- Inform department in advance of all hazardous materials to be used during a project. Inform building occupants upon request by occupants or any employee.
- Supply a copy of all MSDS's for those materials upon request.
- Verify that each container used is labeled in accordance with this HCP.
- A copy of the NBC2 Hazard Communication Program will be available

Appendix A

Chemical Inventory List

Appendix A
Chemical Inventory List

Chemical Name	Manufacturer/supplier

Appendix B

Employee Training Record

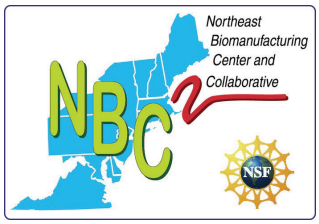
Employee Training Record

Supervisor:	Department:
Unit:	Location:
Date Training Developed:	Training Program Revision Dates:

Employee Name	Job Title	General Haz Com Training (most recent date)	Unit or Dept Specific Haz Com Training (most recent date)

Lab, Clinic, or Unit Specific Hazard Communication Training Provided: List the specific training provided by the PI or supervisor to the individuals listed above. Provide separate descriptions as necessary.
1. Explanation of the Completed Unit Specific Haz Com Plan:
2. Chemical Inventory and specific hazardous chemical each employee may encounter
3. Location and availability of MSDSs; explanation of hazards associated with chemicals or groups of chemicals
4. Labeling system used by unit
5. Specific safety training for each person's duties including specific equipment and its use: **Specific PPE- the types used, its use, location, and limitations**:

6. Procedures for non-routine tasks and emergencies
7. Procedures when working with contractors



Hazard Communication Program Audit Form	Document NBC-HCP-001				
	Effective 14-June-2007				
	REV 1	Page	1	of	2

Written Program

Yes	No	
❑	❑	Department or laboratory has a copy of the Hazard Communication Program.
❑	❑	HazCom program Administrator identified.
❑	❑	Written HazCom program displayed and accessible to employees at all times.
❑	❑	All employees informed of HazCom program location.
❑	❑	HazCom Program reviewed annually

Chemical Inventory

Yes	No	
❑	❑	All chemicals located in the workplace are listed on the chemical inventory.
❑	❑	All employees have access to the chemical inventory list.
❑	❑	Individual has been designated to update Chemical Inventory list.
❑	❑	List is accurately updated when chemical product is received; chemical inventory is checked to ensure chemical is recorded.

MSDS

Yes	No	
❑	❑	MSDS location identified and ALL employees are informed of location.
❑	❑	MSDS arranged in an orderly fashion to ensure ease of location.
❑	❑	Each chemical present has an MSDS.
❑	❑	Employee designated to ensure MSDS are maintained in accordance with 29CFR1910.1200.
❑	❑	MSDS for chemicals no longer being used are kept in a separate location.
❑	❑	MSDS are legible and current.
❑	❑	MSDS for chemicals involved in an exposure incident are kept and present for the 30-year requirement.
❑	❑	MSDS meets informational requirements in accordance with 29CFR1910.1200.

Labeling

Yes	No	
❑	❑	Primary containers contain appropriate labeling information, (Chemical name, name and address of manufacturer, and appropriate warning info).
❑	❑	Secondary containers contain appropriate labeling information.
❑	❑	Employee designated to ensure labels are correct.
❑	❑	Primary and Secondary labels are updated and legible

Training

Yes	No	
❑	❑	All employees receive training in Hazard Communication in accordance with the NBC2 Hazard communication program and 29CFR1910.1200. • Initially, prior to assignment to work • Upon introduction of new hazards (new chemicals, new tasks, etc.) • Upon assignment to non-routine tasks
❑	❑	Training records kept on file by Supervisor / Foreman.
❑	❑	Employee's know what to do in case of an emergency

Contractors

Yes	No	
❑	❑	Outside contractors are informed of NBC2 Hazard Communication policy.
❑	❑	Outside contractors are informed of hazardous chemicals to which they may potentially be exposed.
❑	❑	Outside contractors inform employees of Hazardous Chemicals brought onto property.

Comments:_____
_____
_____

Date:_____
Evaluator:_____

www.MSDSAuthoring.com **For MSDS Writing/Authoring Assistance. All of your MSDS Compliance needs.**
Phone 209-649-3913
Fax 209-234-5931

THIS IS A SAMPLE OSHA MSDS TEMPLATE

Material Safety Data Sheet
May be used to comply with OSHA's Hazard Communication Standard, 29 CFR 1910 1200. Standard must be consulted for specific requirements.

U.S. Department of Labor
Occupational Safety and Health Administration
(Non-Mandatory Form)
Form Approved
OMB No. 1218-0072

IDENTITY *(as Used on Label and List)*

Note: Blank spaces are not permitted. If any item is not applicable or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's name	Emergency Telephone Number
Address *(Number, Street, City, State and ZIP Code)*	Telephone Number for Information
	Date Prepared
	Signature of Preparer *(optional)*

Section II—Hazardous Ingredients/Identity Information

Hazardous Components (Specific Chemical Identity, Common Name(s))	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (optional)

Section III—Physical/Chemical Characteristics

Boiling Point		Specific Gravity (H_20 = 1)	
Vapor Pressure (mm Hg)		Melting Point	
Vapor Density (AIR = 1)		Evaporation Rate (Butyl Acetate = 1)	

Solubility in Water

Appearance and Odor

Section IV—Fire and Explosion Hazard Data

Flash Point (Method Used)	Flammable Limits	LEL	UEL

Extinguishing Media

Special Fire Fighting Procedures

Unusual Fire and Explosion Hazards

(Reproduce locally) OSHA 174 Sept. 1985

Section V—Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable		

Incompatibility *(Materials to Avoid)*

Hazardous Decomposition or Byproducts

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur		

Section VI—Health Hazard Data

Route(s) of Entry	Inhalation?	Skin?	Ingestion?

Health Hazards *(Acute and Chronic)*

Carcinogenicity	NTP?	IARC Monographs?	OSHA Regulated?

Signs and Symptoms of Exposure

Medical Conditions
Generally Aggravated by Exposure

Emergency and First Aid Procedures

Section VII—Precautions for Safe Handling and Use

Steps to Be Taken in Case Material Is Released or Spilled

Waste Disposal Method

Precautions to Be Taken in Handling and Storing

Other Precautions

Section VII—Control Measures

Respiratory Protection *(Specify Type)*

Ventilation	Local Exhaust	Special
	Mechanical *(General)*	Other

Protective Gloves	Eye Protection

Other Protective Clothing or Equipment

Work/Hygienic Practices

Health	3
Fire	0
Reactivity	0
Personal Protection	J

Material Safety Data Sheet
Potassium cyanide MSDS

Section 1: Chemical Product and Company Identification

Product Name: Potassium cyanide

Catalog Codes: SLP3853

CAS#: 151-50-8

RTECS: TS8750000

TSCA: TSCA 8(b) inventory: Potassium cyanide

CI#: Not available.

Synonym:

Chemical Name: Potassium Cyanide

Chemical Formula: KCN

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call:
1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Potassium cyanide	151-50-8	100

Toxicological Data on Ingredients: Potassium cyanide: ORAL (LD50): Acute: 5 mg/kg [Rabbit]. 8.5 mg/kg [Mouse]. 5 mg/kg [Rat].

Section 3: Hazards Identification

Potential Acute Health Effects:
Very hazardous in case of skin contact (irritant, permeator), of eye contact (irritant), of ingestion, of inhalation. Corrosive to eyes and skin. The amount of tissue damage depends on length of contact. Eye contact can result in corneal damage or blindness. Skin contact can produce inflammation and blistering. Inhalation of dust will produce irritation to gastro-intestinal or respiratory tract, characterized by burning, sneezing and coughing. Severe over-exposure can produce lung damage, choking, unconsciousness or death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

Potential Chronic Health Effects:
CARCINOGENIC EFFECTS: Not available.
MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells.
TERATOGENIC EFFECTS: Not available.
DEVELOPMENTAL TOXICITY: Not available.

The substance is toxic to blood, liver.
The substance may be toxic to cardiovascular system, upper respiratory tract, Urinary system, central nervous system (CNS).
Repeated or prolonged exposure to the substance can produce target organs damage. Repeated exposure of the eyes to a low level of dust can produce eye irritation. Repeated skin exposure can produce local skin destruction, or dermatitis. Repeated inhalation of dust can produce varying degree of respiratory irritation or lung damage. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

Section 4: First Aid Measures

Eye Contact:
Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.

Skin Contact:
In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used.Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

Serious Skin Contact:
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

Serious Inhalation:
Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. WARNING: It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.

Ingestion:
If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: Non-flammable.

Auto-Ignition Temperature: Not applicable.

Flash Points: Not applicable.

Flammable Limits: Not applicable.

Products of Combustion: Not available.

Fire Hazards in Presence of Various Substances: Not applicable.

Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of mechanical impact: Not available.
Risks of explosion of the product in presence of static discharge: Not available.
Explosive in presence of oxidizing materials.

Fire Fighting Media and Instructions: Not applicable.

Special Remarks on Fire Hazards: Contact with acids or acid salts causes immediate formation of toxic and flammable hydrogen cyanide gas.

Special Remarks on Explosion Hazards:
Chlorates + potassium cyanide explode when heated.
Potassium cyanide + nitrites may cause explosion.
Nitrogen trichloride explodes on contact with potassium cyanide.
Potassium cyanide + hydrogen cyanide is a frictioin and impact-sensitive explosive and may initiate detonation of liquid hydrogen cyanide.
Mercuric nitrate + potassium cyanide explodes when heated and contained in narrow iignition tubes.
Perchloryl fluoride + potassium cyanide causes an explosive reaction at 100-300 C.
Potassium cyanide + ammoniacal silver, following heating, shock or standing can cause an explosion.
Heating of potassium cyanide & chromium tetraoxide can cause an explosion.
Mixtures of metal cyanides with metal chlorates, perchlorates, or nitrates causes a violent explosion.

Section 6: Accidental Release Measures

Small Spill:
Use appropriate tools to put the spilled solid in a convenient waste disposal container. If necessary: Neutralize the residue with a dilute solution of acetic acid.

Large Spill:
Corrosive solid. Poisonous solid.
Stop leak if without risk. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Neutralize the residue with a dilute solution of acetic acid. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

Section 7: Handling and Storage

Precautions:
Keep locked up.. Keep container dry. Do not ingest. Do not breathe dust. Never add water to this product. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents.

Storage:
Moisture Sensitive. Light Sensitive. Protect from light. Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 24°C (75.2°F).

Section 8: Exposure Controls/Personal Protection

Engineering Controls:
Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.

Personal Protection:
Splash goggles. Synthetic apron. Vapor and dust respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Vapor and dust respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits:
STEL: 5 (mg/m3) from ACGIH (TLV) [United States]
CEIL: 0.7 from NIOSH [United States]
CEIL: 5 (mg/m3) from NIOSH [United States]
Consult local authorities for acceptable exposure limits.

Section 9: Physical and Chemical Properties

Physical state and appearance:
Solid. (Crystalline or Granular solid. Deliquescent solid.)

Odor:
Almond-like. Like bitter almonds. Odor of hydrogen cyanide (Slight.)

Taste: Not available.

Molecular Weight: 65.11 g/mole

Color: White.

pH (1% soln/water): 11 [Basic.]

Boiling Point: 1625°C (2957°F)

Melting Point: 634.5°C (1174.1°F)

Critical Temperature: Not available.

Specific Gravity: 1.553 (Water = 1)

Vapor Pressure: Not applicable.

Vapor Density: Not available.

Volatility: Not available.

Odor Threshold: Not available.

Water/Oil Dist. Coeff.: Not available.

Ionicity (in Water): Not available.

Dispersion Properties: See solubility in water, methanol.

Solubility:
Easily soluble in hot water.
Soluble in cold water.
Partially soluble in methanol.
Very slightly soluble in ethanol.
Partially soluble in glycerol, hydroxylamine, and liquid ammonia.
Very soluble in formamide

Section 10: Stability and Reactivity Data

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Incompatible materials, water, moisture, light,air

Incompatibility with various substances:
Highly reactive with oxidizing agents.
Reactive with acids.

Corrosivity: Non-corrosive in presence of glass.

Special Remarks on Reactivity:
Moisture sensitive.
Air Sensitive.
Deliquescent.
Protect from light.
Reacts with water or any acid releasing hydrogen cyanide.
Toxic gases and vapors (such as hydrogen cyanide and carbon monoxide) may be released when potassium cyanide decomposes.
Incompatible with acids, acid syrups, alkaloids, chloral hydrate, iodine, metallic salts, permanganates, chlorates, peroxides.
Potassium cyanide may react with carbon dioxide in ordinary air to form toxic hydrogen cyanide gas.
Potasssium cyanide is readily oxidized by heating to potassium cyanate in presence of oxygen or easily reduced oxides.

Special Remarks on Corrosivity: Not available.

Polymerization: Will not occur.

Section 11: Toxicological Information

Routes of Entry: Absorbed through skin. Dermal contact. Inhalation. Ingestion.

Toxicity to Animals: Acute oral toxicity (LD50): 5 mg/kg [Rat].

Chronic Effects on Humans:
MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells.
Causes damage to the following organs: blood, liver.
May cause damage to the following organs: cardiovascular system, upper respiratory tract, Urinary system, central nervous system (CNS).

Other Toxic Effects on Humans: Very hazardous in case of skin contact (irritant, permeator), of ingestion, of inhalation.

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans:
May cause adverse reproductive effects (female fertility and fetotoxicity).
May affect genetic material.

Special Remarks on other Toxic Effects on Humans:
Acute Potential Health Effects:
Skin: May be fatal if absorbed through skin. Causes skin irritation and possible burns especially if the skin is wet or moist. May be absorbed through skin and cause symptoms similar to those described for ingestion.
Eyes: Causes eye irritation and possible eye burns.
Inhalation: May be fatal if inhaled. Causes respiratory tract and mucous membrane irritation. Inhalation of high concentrations may cause central nervous system effects similar to those described for ingestion.
Ingestion: May be fatal if swallowed. Causes severe gastrointestinal tract irritation with nausea, vomiting and possible burns. May cause tissue anoxia. May affect behavior/Central Nervous system, Metabolism, cardiovascular system, respiratory system, blood, respiration. Symptoms of cyanide poisoning may include flushing, nausea, vomiting, palpitations, tachycardia, hypotension, hypertension, increased pulse rate, arrhythmias, heart conduction defects, hypernea, headache, dizziness, confusion, anxiety, agitation, tremors, weakness, hyperventilation, dyspnea, apnea, severe hypoxic signs in absence of cyanosis (cyanosis is generally late finding), convulsions, seizures, memory loss, insomnia, metabolic acidosis, poor appetite.

Chronic Potential Health Effects:
Skin: Prolonged or repeated skin contract may cause dermatitis.
Ingestion: Prolonged or repeated exposure from ingestion may affect the urinary system, brain, liver and thyroid (goiter) as well have the same effects as acute overexposure.

Section 12: Ecological Information

Ecotoxicity: Not available.

BOD5 and COD: Not available.

Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

Toxicity of the Products of Biodegradation: The products of degradation are less toxic than the product itself.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14: Transport Information

DOT Classification: CLASS 6.1: Poisonous material.

Identification: : Potassium cyanide UNNA: 1680 PG: I

Special Provisions for Transport: Marine Pollutant

Section 15: Other Regulatory Information

Federal and State Regulations:
Connecticut hazardous material survey.: Potassium cyanide
Illinois chemical safety act: Potassium cyanide
New York acutely hazardous substances: Potassium cyanide
Rhode Island RTK hazardous substances: Potassium cyanide
Pennsylvania RTK: Potassium cyanide
Minnesota: Potassium cyanide
Massachusetts RTK: Potassium cyanide
Massachusetts spill list: Potassium cyanide
New Jersey: Potassium cyanide

Other Regulations:
OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).
EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

Other Classifications:

WHMIS (Canada):
CLASS D-1A: Material causing immediate and serious toxic effects (VERY TOXIC).
CLASS E: Corrosive solid.
WHMIS Class B-6: Reactive and very flammable material.

DSCL (EEC):
R16- Explosive when mixed with oxidizing substances.
R28- Very toxic if swallowed.
R38- Irritating to skin.
R40- Possible risks of irreversible effects.
R41- Risk of serious damage to eyes.
S1/2- Keep locked up and out of the reach of children.
S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28- After contact with skin, wash immediately with plenty of [***]
S36/37- Wear suitable protective clothing and gloves.
S39- Wear eye/face protection.
S45- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S46- If swallowed, seek medical advice immediately and show this container or label.

HMIS (U.S.A.):

Health Hazard: 3

Fire Hazard: 0

Reactivity: 0

Personal Protection: j

National Fire Protection Association (U.S.A.):

Health: 3

Flammability: 0

Reactivity: 0

Specific hazard:

Protective Equipment:
Gloves.
Synthetic apron.
Vapor and dust respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate.
Splash goggles.

Section 16: Other Information

References: Not available.

Other Special Considerations: Not available.

Created: 10/11/2005 01:51 PM

Last Updated: 10/11/2005 01:51 PM

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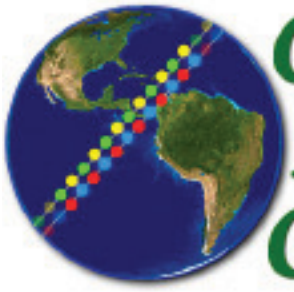
Competencies Master List	QC CHEM	QC MICRO	MANUF UP	MANUF DOWN	MAINT
Regulatory, Safety, and Environmental Compliance (CONTINUED)					
Safety					
Understand and apply basic methods for the safe handling, transport, and storage of biological and chemical materials.	x	x	x	x	x
Understand and apply basic methods for handling hazardous waste.	x	x	x	x	x
Understand and apply Lock-out/Tag-out and line breaking procedures.	x	x	x	x	x
Understand Fall protection (heights).	x	x	x	x	x
Understand and apply Confined space entry methods.	x	x	x	x	x
Understand and use Personal Protective Equipment as required.	x	x	x	x	x
Understand the purpose and procedures associated with Safety audits.	x	x	x	x	x
Understand and apply OSHA regulations.	x	x	x	x	x
Understand basic disinfection and sterilization methods.	x	x	x	x	x
Participate in emergency drills and response teams.	x	x	x	x	x
Identify hazards in the workplace.	x	x	x	x	x
Identify spills/leakages.	x	x	x	x	x
Identify emergency equipment.	x	x	x	x	x
Understand and locate information in an MSDS.	x	x	x	x	x
Understand different biosafety levels.	x	x	x	x	x
Understand rationale behind machine grounding and use of fail-safe devices.	x	x	x	x	x
Environmental					
Understand environmental regulations and plant waste processing systems.	x	x	x	x	x

Quality Assurance



GLOBAL
BIOMANUFACTURING
CURRICULUM

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GMP POPCORN EXERCISE CHECKLIST

Goal:
Make a batch of GMP popcorn within a specified timeframe (1.5 hours).

Objective:
Understand the complexity of a GMP process.
Gain appreciation of teamwork and cooperation of all departments.

How it mirrors industry:

- You will be frustrated at times
- You will be rushed.
- You will feel a sense of accomplishment once you made the batch!
- The actual time for the chemistry / fermentation / etc. is very small compared to the time it takes to get all GMP documentation in place.

Supplies:

1. Department tasks and deliverables
2. Approved labels
3. Quarantine labels
4. Box for approved and quarantine
5. Microwave Popcorn
6. Access to photocopier
7. Access to microwave
8. Water in a squirt bottle
9. 409 or other type of cleaning agent
10. Paper towels
11. Sponge
12. Measuring cups
13. Bags for the finished product

Teams:
Material Control: 2 people
QC: 2 people that like to test material
QA: 2 people that have an eye for written details
Production: 4 people that like to WORK

QUALITY ASSURANCE

ROLE OF QA:

- Review and Approval all quality related documents.
- Issue all controlled documents.
- Provide oversight on the production campaign.
- Disposition Raw materials and final products.

Checklist of Items to Accomplish:

- Review and Approve Raw Material Master Specification Sheet
- Review and Approve Final product Master Specification Sheet
- Review and Approve Master Batch Record
- Approve actual Raw Materials for use
- Inspect Microwave for Cleanliness
- Review completed Batch Record after production is complete
- Review QC data and Approve actual Popcorn

Your Tasks are:

1. APPROVING MASTER SPECIFICATIONS:

Production will be submitting for your review AND approval:

1. **RAW MATERIAL SPECIFICATION SHEET** for the KERNELS
2. **FINAL PRODUCT SPECIFICATION SHEET** for the POPCORN.

Instructions: *Review to ensure all boxes that are **shaded** have been completed. All information provided should make sense and be reasonable. Try where possible to have the team write in quantitative specifications (i.e. "No more than 2 dark pieces of popcorn."). If you don't agree with the specifications, or have questions, feel free to go back to the Production Team for clarification.*

HOW TO APPROVE A MASTER SPECIFICATION SHEET:

To make the **specification sheet** effective complete the following:

a). **Sign** your name in the shaded box for "QA Approval".

b). **Write** in an **effective date** (i.e. today's date) in the upper right hand corner.

c). Make a **photocopy** (yes, go to the photocopier) of the original document you just signed and give a copy to QC.

d). **File** the original in QA.

2. APPROVING A MASTER BATCH RECORD:

Production will be submitting for your review:

1. A Master **BATCH RECORD** to make the POPCORN.

Instructions: *Review to ensure all boxes that are **shaded** have been*

Johnson Matthey Pharmaceutical Materials, Inc.
25 PATTON ROAD, DEVENS, MA 01432 TEL: 978.784.5000 FAX: 978.784.5500 WWW.PHARMECO.COM

completed. All information provided should make sense and be reasonable. HINT: The production team should have quantitative numbers in the process (i.e. Pop popcorn for 1.5 – 2.5 minutes). If you don't agree with the process description or have questions feel free to go back to the Production Team for clarification.

HOW TO APPROVE A MASTER BATCH RECORD:

To make the BATCH RECORD effective complete the following:

a). **Sign** your name in the shaded box for "QA Approval" on the front page and initial the bottom of all pages in the QA box designated.

b). **Write in** an **effective date** (i.e. today's date) in the upper right hand corner on ALL PAGES.

c). Make a **photocopy** of the Master Batch Record

d). Write in the **Lot Number** on all pages (in the box for LOT NUMBER) **ON THE PHOTOCOPY OF THE BATCH RECORD**. (See below for instructions):

INSTRUCTIONS FOR ASSIGNING A UNIQUE LOT NUMBER:

Lot number should be POP-YEAR-001. For example: POP-03-001

e). **File** the original batch record in QA.

f). **Give the photocopy** of the batch record to production. (*We call this "Issuing a batch record to production*")

3. APPROVING RAW MATERIALS:

Before production can use the kernels in their production run they must be approved by QC AND QA! Follow the instructions below to approve the raw materials (i.e. Kernals).

HOW TO APPROVE RAW MATERIALS:

1. Once QC finishes the "testing" on the Kernals, **review** the information written in on the specification sheet and the accompanying documentation to ensure no empty spaces.
2. If acceptable, sign your name in the box for QA at the bottom of the page on the **specification sheet** and check off "**Approved**".
3. Fill in the spots on the **Approval labels. Make one for each container / package PLUS 1 extra for the receiving report.**
4. Complete the remaining boxes designated "QA" on the **Receiving report**. Place your extra label on the receiving report.
5. Give **approval labels** to Material Control.

4. INSPECTING EQUIPMENT:

Before production can use a piece of equipment it must be clean! It is a common practice in industry to have QA inspect the equipment after production cleans it.

HOW TO INSPECT EQUIPMENT:

1. Production will be requesting QA to **visually inspect** the microwave for cleanliness. If it is not satisfactorily clean, have Production re-clean the microwave.

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2. Once acceptable, sign/date the **Cleaning Log** in the spot for "QA initials"

5. INTERNAL AUDITING:

Feel free to audit the production area during production!

6. REVIEWING COMPLETED PRODUCTION BATCH RECORDS:

Once production of the popcorn is complete, the Production team will be submitting the completed batch record for your review. Before the popcorn is "Dispostioned" QA must review the completed batch record AND completed Quality Control testing. BOTH items must be satisfactorily before the popcorn can be approved!

HOW TO REVIEW A COMPLETED BATCH RECORD:

1. Ensure all information is recorded and completed per requirements of the batch record.
2. If any items were not completed –return to production for correction.
3. The team must not of deviated from requirements in the batch record. (i.e: if it says to "Pop the popcorn in the microwave for 2-3 minutes they must not go over 3 minutes or under 2 minutes without some justification.)
4. Once you are satisfied with the completed record, sign your name in the "Reviewed by QA" box
5. DO NOT YET APPROVE THE BATCH. YOU NEED THE QC DATA FIRST.

7. REVIEWING COMPLETED QC DATA

Once the QC data is complete – review the QC information for completeness. If both the Batch Record and QC data are acceptable you may sign both documents as "APPROVED". NOW THE POPCORN IS APPROVED.

HOW TO APPROVE QC DATA ON POPCORN

1. Once QC finishes the "testing" on the Popcorn, **review** the information written in on the specification sheet and the accompanying documentation to ensure no empty spaces.
2. If acceptable, sign your name in the box for QA at the bottom of the page on the **specification sheet** and check off "**Approved**".
3. If the batch record is complete and the QC data is complete you can now fill out the **Approval labels!**
4. Complete the remaining boxes designated "QA" on the **Receiving report**. **Make one label for each container / package PLUS 1 extra for the receiving report.**
5. Place your extra label on the receiving report.
6. Give **approval labels** to Material Control.

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M A T E R I A L C O N T R O L

ROLE OF MATERIAL CONTROL:

- MC controls the flow of materials and limits the access of materials to prevent against "off-grade" materials being used in production.
- Material Control inspects all incoming materials/packages for integrity.
- Material control offers expertise in packaging and storing of materials.

Your Tasks are:

1. **STORAGE AREAS**:

Designate two separate areas: "**Quarantine"** and "**Approved."** Use the boxes provided and label them appropriately.

2. **Inspect** all incoming packages for possible signs of damage during shipping.

3. **RECEIVING RAW MATERIALS:**

You will receive raw materials to enter into your system.

Instructions:

1. By using information on the package - fill in **SECTION 1** on the "**Receiving Report**". Use the template provide.
2. Give a **"RECEIVING NUMBER"** to the material. Use the following format: DDMMYY – 000
   (i.e. for the first item received on January 2, 2007 write: 010207-001)
3. Write this receiving number on the Receiving Report. Attach the COA to the Receiving Report.
4. Complete a **Quarantine Label** and place the completed **"Quarantine"** label on the material and transfer to the **"Quarantine" area**.
5. Give the "**Receiving Report**" and COA to QC- this is their cue to sample the material and begin testing.

4. **LABELING RAW MATERIALS APPROVED:**

1. QA will give Material Control "Approval" labels to apply to the material that is in "Quarantine".
2. Place the Approval Label to cover up the word "QUARANTINE" on the quarantine label. Move the material to the "Approved" area.
3. Production may now have the material. Give only "Approved" material to production for their use.

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5. RECEIVING FINAL PRODUCTS INTO QUARANTINE:

You will need to take the final product (i.e. popcorn) and place it in quarantine.

Instructions:

1. fill in **SECTION 1** on the "**Receiving Report**". Use the template provide. Ask production team for lot number.
2. Complete a **Quarantine Label** and place the completed **"Quarantine**" label on the material and transfer to the **"Quarantine" area**.
3. Give the "**Receiving Report**" to QC- this is their cue to sample the material and begin testing.
4. QA will give Material Control the "Approval" labels to apply to the material that is placed in "Quarantine". Move the material to the "Approved" area.

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P R O D U C T I O N

ROLE OF PRODUCTION:

- Execute the process according the batch record to produce a product within specifications
- Coordinate the batch record, release of raw materials, and equipment

Checklist of Items to Accomplish:

- ❑ Write Master Raw Material Specification Sheet and circulate for approvals
- ❑ Write Master Final Product Specification Sheet and circulate for approvals
- ❑ Write Master Batch Record and circulate for approvals
- ❑ Ensure Raw Materials have been tested and approved
- ❑ Clean Microwave per SOP
- ❑ MAKE GMP Popcorn! And complete batch record as you go!

HINT: PRODUCTION HAS MANY DOCUMENTS TO WRITE ….IT IS BEST IF YOU MULTI TASK AND SPREAD THE WORK AMONG THE DEPARTMENT.

Your Tasks are:

1. WRITING MASTER SPECIFICATIONS:

Production must write the following specifications and give to QC and QA to review and approve:

1. **RAW MATERIAL SPECIFICATION SHEET** for the KERNELS
2. **FINAL PRODUCT SPECIFICATION SHEET** for the POPCORN.

Instructions:

Use the template in your package. *Neatly complete all boxes (i.e. Vendor, storage conditions, specifications, etc.) that are* ***shaded*** *(except the signatures). All information provided should make sense and be reasonable. Try where possible to write quantitative specifications (i.e. "No more than 2 dark pieces of popcorn).*

1. Circulate for signatures –
   - 1st: Production signer in "Written By"
   - 2nd: Production Supervisor signs
   - 3rd: QC Supervisor
   - 4th: QA

NOTE: QA will keep the final document.

2. WRITING A MASTER BATCH RECORD:

Production must write a Master Batch Record for the production of Popcorn.
Use the Template provided.

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Instructions:
*Enter all the information in the boxes that are **shaded.** You must describe what you think your process will be. All information provided should make sense and be reasonable.*

HOW TO WRITE A MASTER BATCH RECORD:

a). Complete all shaded areas. Sign your name on the front page

b). Give to Production supervisor for review. Sign your name on the front page.

c). Give to QA to review. Sign your name on the front page.

d). Once QA is happy with the batch record, they will issue you a copy to conduct your production.

3. Don't forget to check with QC regarding the testing of your **raw materials (i.e.: Kernels)**.

4. CLEANING EQUIPMENT:

Before production can use a piece of equipment it must be clean! It is a common practice in industry to have QA inspect the equipment after production cleans it.

HOW TO CLEAN EQUIPMENT:

1. Use the SOP provided to clean your equipment (i.e. Microwave).
2. Once the production operator has cleaned the Equipment, complete the documentation required on the Cleaning log and have the Supervisor inspect the equipment.
3. Request QA to **visually inspect** the microwave for cleanliness.
4. Once QA has inspected the equipment and found it acceptable, you may now use the equipment.

5. STARTING PRODUCTION!

1. Once you have the issued batch record from QA; APPROVED raw material; and CLEAN equipment, **you may start production of the POPCORN.**
2. Follow the process in your batch record and document as you go. Once the production of the popcorn is complete so should your batch record.

ENDING PRODUCTION:

1. Notify Material Control to remove your popcorn from the equipment and place it in quarantine.
2. While QC is testing the material, the Production Operator must review the record to ensure all information is complete.

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3. Production supervisor must review and sign the back of the batch record.
4. Submit to QA for review.
5. Clean the equipment as documented above.
6. Wait to hear from QA if your material is approved!

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QUALITY CONTROL

ROLE OF QC:

- Test all materials to be used by Production.

Checklist of Items to Accomplish:

- Review Raw Material (i.e Kernals) Specification Sheet
- Test Raw Materials
- Review Final Product (i.e. Popcorn) Specification Sheet
- Test Final Product Materials

Your Tasks are:

1. APPROVING MASTER SPECIFICATIONS:

Production will be submitting for your review AND approval:

1. **RAW MATERIAL SPECIFICATION SHEET** for the KERNELS
2. **FINAL PRODUCT SPECIFICATION SHEET** for the POPCORN.

Instructions: *Review to ensure all boxes that are **shaded** have been completed. All information provided should make sense and be reasonable. Try where possible to have the team write in quantitative specifications (i.e. "No more than 2 dark pieces of popcorn). If you don't agree with the specifications or have questions feel free to go back to the Production Team for clarification.*

HOW TO APPROVE A MASTER SPECIFICATION SHEET:

To approve the **specification sheet** complete the following:

a). **Sign** your name in the shaded box for "QC Approval".
b). Give to QA for their review.

2. DOCUMENTS NEEDED:

NOTE: QA will give you copies of the specification sheets to document the results of the testing for BOTH the kernels and the popcorn.

3. APPROVING RAW MATERIALS:

Before production can use the kernels in their production run they must be approved by QC AND QA! Follow the instructions below to test and approve the raw materials (i.e. Kernals).

HOW TO TEST AND APPROVE RAW MATERIALS:

1. Material Control will be giving you a **RECEIVING REPORT** for both **KERNALS** and **POPCORN** (once made). This is your cue that something is in quarantine and needs to be tested by QC. Take your

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specification sheet to quarantine and begin to following the sampling and testing instructions.

2. Once the analyst completes the "testing" on the Kernals, **review** the information written in on the specification sheet and the accompanying documentation to ensure no empty spaces. The analyst will sign his/her initials and date in the column.
3. The QC Supervisor will review the package of data the analyst completed and If acceptable, sign your name in the box for QC approval at the bottom of the page on the **specification sheet** and check off "**Approved**".
4. Give package to QA.

4. **APPROVING FINAL PRODUCT (I.E: Popcorn)**:

Once production has made the popcorn, you will be required to test the material. Follow the instructions below to test and approve the final product (i.e. popcorn).

HOW TO TEST AND APPROVE FINAL PRODUCTS:

1. Material Control will be giving you a **RECEIVING REPORT** for the **POPCORN** (once made). This is your cue that something is in quarantine and needs to be tested by QC. Take your specification sheet to quarantine and begin to following the sampling and testing instructions.
2. Once the analyst completes the "testing" on the Popcorn, **review** the information written in on the specification sheet and the accompanying documentation to ensure no empty spaces. The analyst will sign his/her initials and date in the column.
3. The QC Supervisor will review the package of data the analyst completed and If acceptable, sign your name in the box for QC approval at the bottom of the page on the **specification sheet** and check off "**Approved**".
4. Give package to QA.

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Batch Production Record

Product Name: Popcorn

Effective Date:

Revision: 0

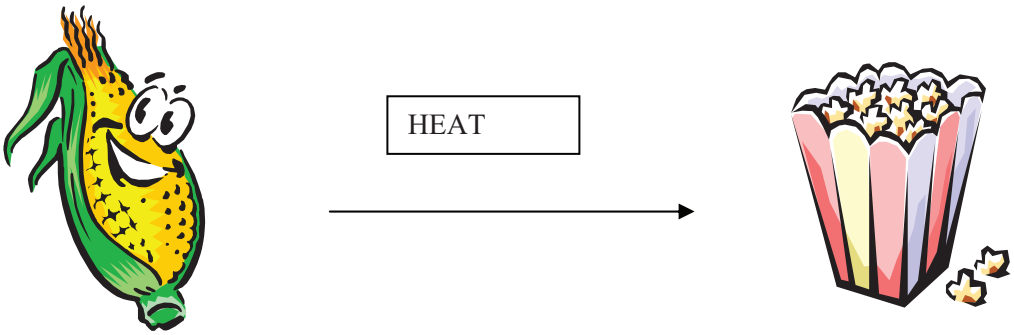
Written By:		**Date:**	
Production Supervisor Approval:		**Date:**	
Quality Assurance Approval:		**Date:**	

INPUT MATERIALS:

INPUT MATERIALS	PRODUCTION OPERATOR	CHECKED BY

Note:
Input Materials are materials used in your process (i.e. Kernals).

REACTION SCHEME



EQUIPMENT CHECKLIST

(Standard batch size)

		Inventory Number	Production Operator Initial and date
	Microwave Oven		

MANUFACTURING DIRECTIONS FOR LAB SCALE BATCH:

(completed by QA when issued)

Lot Number:

(Completed by QA on Master)

Effective Date:

Revision: 0

COMPOUND: POPCORN

PROCEDURE

Date:

		Operator init./time	Coworker init./time
1.			
2.			
3.			
4.			
5.			
6.			

Document Approval

Quality Assurance Initial:	

MANUFACTURING DIRECTIONS FOR LAB SCALE BATCH:

(completed by QA when issued)

Lot Number:

(Completed by QA on Master)

Effective Date:

Revision: 0

COMPOUND: POPCORN

Date:

		Operator init./time	Coworker init./time
7.			
8.			
9.			
10.			

Comments: (initial and date any comments)

Document Approval

Quality Assurance Initial:	

SUMMARY OF RESULTS

Actual Yield %:	%
Operator:	Supervisor:

STORAGE OF MATERIAL

Total containers of product transferred to storage:

STORAGE CONDITIONS: Store at room temperature.

CONCLUSION

Date Production Finished:

Production Operator Initials:

Production Supervisor Approval:

Date:

Quality Assurance Reviewer:

Date:

DISPOSITION OF LOT # _____. ___. ____.

Approved

Non-Conforming

Quality Assurance Signature:

Date:

Document Approval

Quality Assurance Initial:

RECEIVING REPORT

SECTION I : RECEIVING (Completed by Material Control)	
Material Name:	**Date Received:**
Supplier:	**Receiving Lot No.:**
Quarantine Label Applied: Yes ☐	**Number of Containers:**
Completed by:	**Date:**

SECTION II: SAMPLING and INSPECTION (Completed by QC)			
Total sample quantity (if final product otherwise N/A):	**Number of Containers Sampled / Inspected:**	**By:**	**Date:**

SECTION III: LABELING (Completed by QA)		
Number of labels issued: (QA)	**By**	**Date:**
Number of sample labels: (QA)	**By**	**Date:**
Attach Sample Label Below (QA)	**By:**	**Date**

Place sample label below:

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RAW MATERIAL SPECIFICATION SHEET

Receiving Number:		**Revision:**	**Effective Date:**

Item Description: KERNELS	
Structure: 	**Written By:** **Date:** **QC Approval:** **Date:** **Production Supervisor Approval:** **Date:** **QA Approval:** **Date:**
Hazards:	**MAY HARM TEETH!**

Storage Condition:		**Supplier:**	

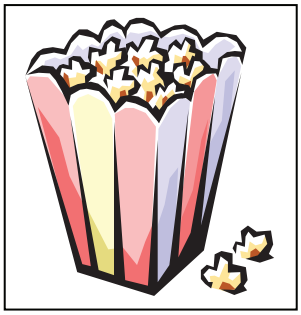
SPECIFICATIONS AND RESULTS

Test	Method	Specification	Result	QC Analyst Init. / Date
Physical Description	**Visual**			

QC Approval	❑ **Approved** ❑ **Non-Conforming**	**Date:**
QA Approval	❑ **Approved** ❑ **Non-Conforming**	**Date:**

FINAL PRODUCT SPECIFICATION SHEET

Lot Number:		Revision:	Effective Date:

Item Description: POPCORN		
Structure: 	Written By: Date:	
	QC Approval: Date:	
	Project Team Approval: Date:	
	QA Approval: Date:	
Hazards:		

Storage Condition:		Supplier:	

SPECIFICATIONS AND RESULTS

Test	Method	Specification	Result	QC Analyst Init./Date
Physical Description	Visual			

QC Approval	❑ Approved ❑ Non-Conforming	Date:
QA Approval	❑ Approved ❑ Non-Conforming	Date:

Receiving/Lot#:________________
Initials.:_______ Date:_________
Storage Conditions:_____________
QUARANTINE

Receiving/Lot#:________________
Initials.:_______ Date:_________
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QUARANTINE

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Receiving/Lot#:________________
Initials.:_______ Date:_________
Storage Conditions:_____________
QUARANTINE

Receiving/Lot#:________________
Initials.:_______ Date:_________
Storage Conditions:_____________
QUARANTINE

STANDARD OPERATING PROCEDURE

Procedure:	Procedure No.: CLN-0001	Revision: 0
PROCEDURE FOR THE PRODUCT CHANGEOVER CLEANING OF A MICROWAVE OVEN	Effective Date: 01/15/07	
	Replaces Document: New	
Written by: Dept.: Production Date: 01/10/07	Dept. Approval: Dept.: Engineering Date: 01/10/07	
Content Review: Dept.: Engineering Date: 01/10/07	QA Approval: Date: 01/13/07	

I. PURPOSE

This procedure is to ensure proper cleaning of equipment.

III. PROCEDURE

		RESPONSIBILITY
3.1	**Product Changeover Cleaning**	
3.1.1	Enter the lot number of the batch on the "Equipment Cleaning Log".	Production
3.1.2	Clean the equipment using water as a cleaning agent and paper towels as cleaning implements.	Production
3.1.3	First wipe the interior top of the microwave from back to front.	Production
3.1.4	Wipe the interior back of the microwave from top to bottom.	Production
3.1.5	Wipe the interior sides of the microwave from back to front, then top to bottom.	Production
3.1.6	Wipe the interior bottom of the microwave from back to front. If the microwave has a turntable inside, remove pieces and clean them using water as a cleaning agent. Visually inspect for contaminants. Replace them in the microwave when complete.	Production
3.1.7	Complete the "Cleaning Log". Enter the SOP number used, cleaning agent, and time / date of person performing cleaning.	Production
3.1.8	Visually inspect for contaminants. If contaminants are present, repeat steps 3.1.2 through 3.1.7. If acceptance, initial column.	Supervisor
3.1.9	Notify QA to Inspect the equipment and verify that it is clean. Quality Assurance will sign the log in the "QA Initials Column" and check the product changeover box.	Production QA

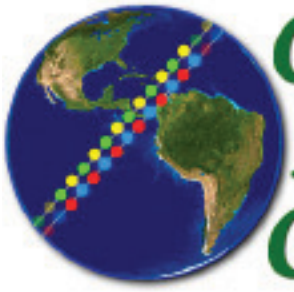
EQUIPMENT CLEANING LOG

Equipment Name: Microwave ____________ Make.: ____________

Lot Number of batch in production	Time/Date of Cleaning	1. SOP No. 2. Solvents Used	Initials of person performing cleaning and a coworker verifying	Cleaning Status
Lot Number: ____________	**Time:** ____________ **Date:** ____________	SOP # ____________ Solvent: ____________	Production Initials: ____________ Supervisor Initials: ____________	❑ Product Changeover QA Initials:______ Date:______
Lot Number: ____________	**Time:** ____________ **Date:** ____________	SOP # ____________ Solvent: ____________	Production Initials: ____________ Supervisor Initials: ____________	❑ Product Changeover QA Initials:______ Date:______
Lot Number: ____________	**Time:** ____________ **Date:** ____________	SOP # ____________ Solvent: ____________	Production Initials: ____________ Supervisor Initials: ____________	❑ Product Changeover QA Initials:______ Date:______

Quality Assurance Competencies List	QC CHEM	QC MICRO	MANUF UP	MANUF DOWN	MAINT
Regulatory, Safety, and Environmental Compliance					
Regulatory (cGMP, FDA, etc..)					
Apply and understand batch records and related documentation.	x	x	x	x	x
Understand rationale and methodology for change control when revising documents or procedures.	x	x	x	x	x
Understand SOP writing practices.	x	x	x	x	x
Use of SOPs.	x	x	x	x	x
Understand the consequences of non-compliance.	x	x	x	x	x
Apply GMP documentation practices for recording data including not back dating documents, not forging, proper time/date format, documentation from source, and not falsifying data.	x	x	x	x	x
Ensure that all batch record steps are signed and verified by a present verifier.	x	x	x	x	x
Ensure that most recent version of SOP, batch record, or other document is used.	x	x	x	x	x
Understand working in a GLP environment.	x	x	x	x	x
Knowledge of typical types of documentation related to facilities and equipment. Examples include maintenance logs, calibration certificates, out of tolerence reports, and installation reports.	x	x	x	x	x
Knowledge of electronic documentation (control and data capture storage systems) practices.	x	x	x	x	x
Knowledge of the FDA 6 systems concept.	x	x	x	x	x
Knowledge of use and history of key regulatory guidance documents including 21 CFR Part 11, 210, 211, 600 Subparts A-D, Sterile Drug Products Prod. By Aseptic Proc., FDA Guidance August 2003, EC Guide on Good Mfg. Practice written by the European Commiss	x	x	x	x	x
Knowledge and use of the United States and European Pharmacopoeia.	x	x	x	x	
Knowledge of ISA and BPE standards.			x	x	x
Principles of ISO standards related to maintenance and repair functions			x	x	x

QC Microbiology



GLOBAL
BIOMANUFACTURING
CURRICULUM

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Title: Operation of MetOne Laser Particle Counter SOP

Approvals:
Preparer: _Bob O'Brien_____ Date _____14Mar07_____
Reviewer: ___Deb Audino_____Date _____14Mar07_____

1. **Purpose:**
   1.1. Operation of the MetOne Laser Particle Counter.
2. **Scope:**
   2.1. Applies to the MetOne Laser Particle Counter for performing sampling of air quality.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. MetOne Model 227 Particle Counter Operator's Manual
   4.2. DPU-414 Printer Manual
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. MetOne Unit
   7.2. Isokinetic Probe
   7.3. DPU-414 Printer
   7.4. Printer Cable
   7.5. MetOne Battery Charger
   7.6. Printer Battery Charger
8. **Procedure:**
   8.1. **Operation**
      8.1.1. Attach the printer to the MetOne unit with the cable if necessary.
      8.1.2. Turn on the printer (on/off switch is on the left hand side) and verify that the "on" light and "online" light are illuminated.
      8.1.3. Remove the red inlet cap from the sensor inlet tube and attach the isokinetic probe. Refer to Figure 1.
      8.1.4. Turn on the MetOne unit using the switch on the bottom of the unit.
      8.1.5. Press [2] for [DSP].
      8.1.6. Press [PROG].
      8.1.7. Press [2] for [INC] or [3] for [DEC] to increase or decrease the location number. The three bench areas with the "P" label is on wheels making them portable, so they may not always be in the same location as shown in the diagram. See figure 4 to determine the appropriate location numbers.
      8.1.8. Press [4] for [RET] to return to the main screen.
      8.1.9. Place the MetOne unit on the bench or cart at the appropriate location facing into the center of the room.

Title: Operation of MetOne Laser Particle Counter SOP

8.1.10. Press [1] for [RUN]. The MetOne unit will count particles until [1] for [STOP] is pressed or until the end of the count cycle (1 minute). One minute will collect 0.1 cubic foot of air. The printer will also automatically print the results.
8.1.11. Press [4] for [RET] to return to the main screen.
8.1.12. Repeat steps 8.1.5 through 8.1.12 until all locations have been tested.
8.1.13. Remove the paper printout.
8.1.14. Turn off the MetOne unit and printer.
8.1.15. Remove isokinetic probe and replace red inlet cap on sensor inlet tube.
8.1.16. Store unit.
8.1.17. Determine the number of particles in $1m^3$ of air by dividing the value printed by 0.002832. Record this value on the printout.

8.2. **Recharging the MetOne battery**
8.2.1. When the "low battery" display appears on the MetOne unit, the battery needs to be recharged.
8.2.1.1. Turn off the MetOne unit and connect the AC adapter to the unit.
8.2.1.2. Plug in the AC adapter and charge the battery for 10-12 hours.

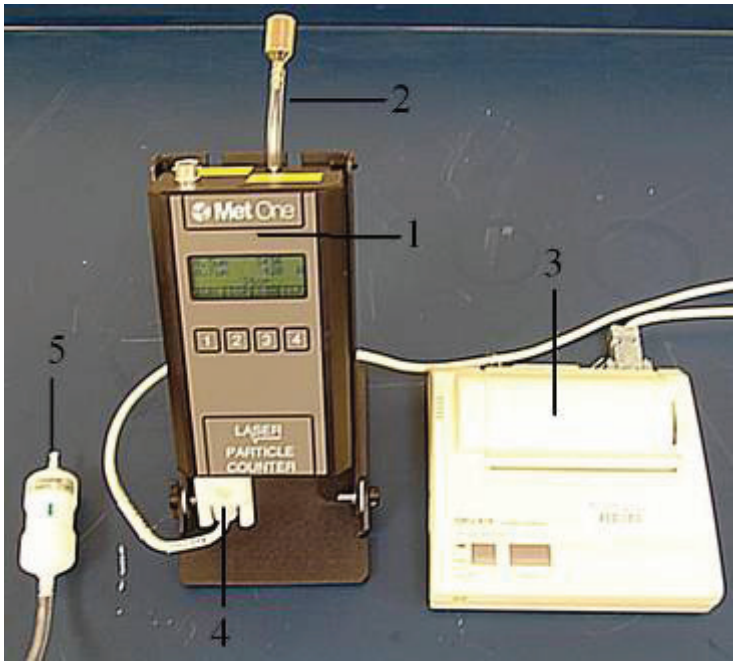
8.3. **Recharging the printer battery**
8.3.1. Turn the printer power OFF.
8.3.2. Connect the AC adapter to the printer.
8.3.3. Plug in the AC adapter and charge the battery for 10-12 hours. The power light will blink while it is charging. The power light goes off when it is fully charged.

9. Attachments:
9.1. Figure 1: MetOne Setup
9.2. Figure 2: Top View of MetOne
9.3. Figure 3: Bottom View of MetOne
9.4. Figure 4: Diagram of Biomanufacturing and Prep Suites

10. History:

Name	Date	Amendment
Bob O'Brien	19Jul05	Initial Release
Bob O'Brien	14Mar07	Update date format, effective date, and diagram of biomanufacturing lab to reflect portable benches.

Title: Operation of MetOne Laser Particle Counter SOP



1. MetOne Unit
2. Isokinetic Probe
3. Printer
4. RS232 Cable
5. Purge Filter
   (Not used in basic operation)

Figure 1: MetOne Setup

Figure 2: Top View of MetOne

Figure 3: Bottom View of MetOne

Title: Operation of MetOne Laser Particle Counter SOP

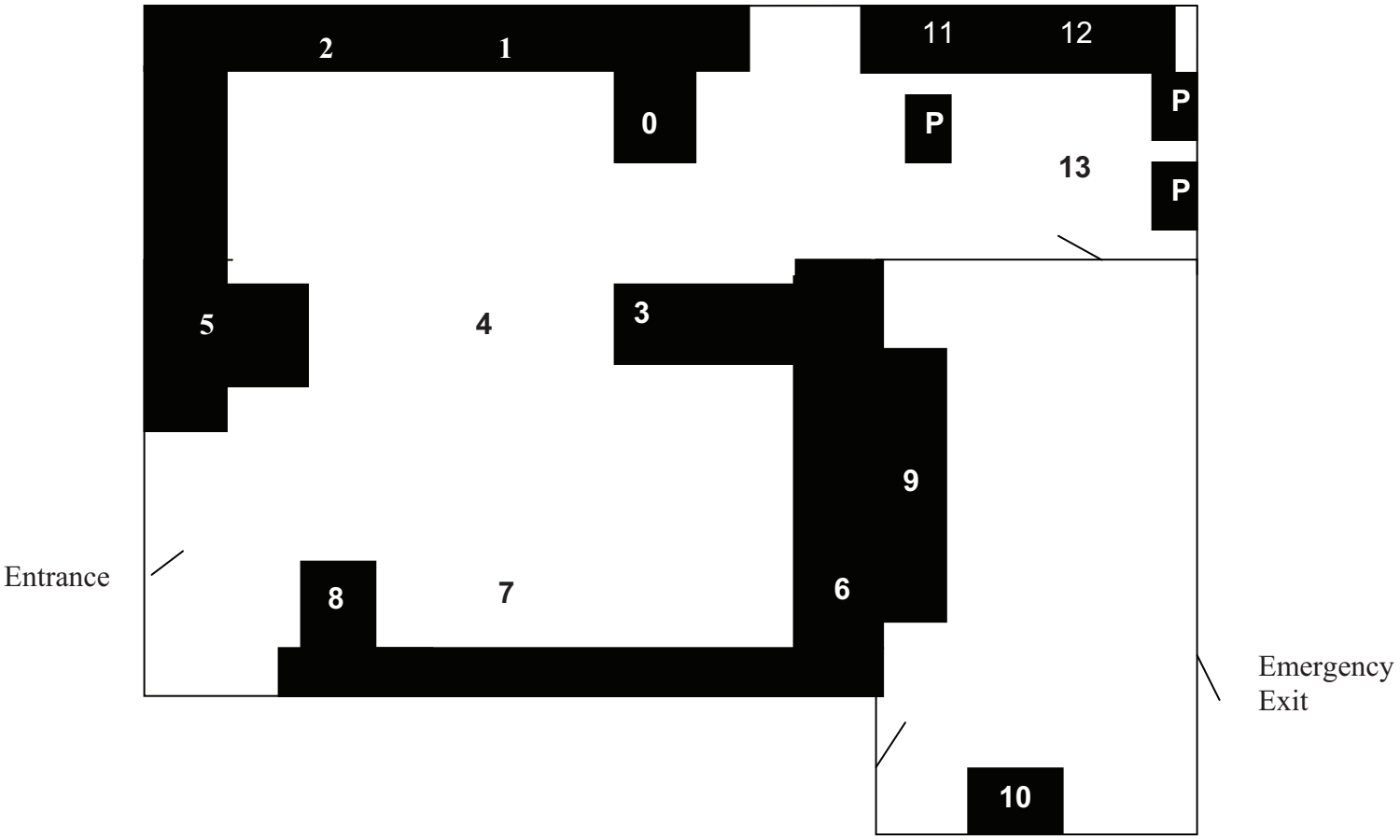


Figure 4: Diagram of Biomanufacturing and Prep Suites

Title: M Air T Millipore Air Tester SOP

Approvals:
Preparer: ___Bob O'Brien _____ Date __14Mar07_____
Reviewer: __ Deb Audino _____ Date __14Mar07_____

1. **Purpose:**
   1.1. The purpose of this SOP is to describe the procedure in using the M Air T Millipore Air Tester in conducting airborne microbial testing.
2. **Scope:**
   2.1. The scope of this SOP is limited to performing airborne microbial testing using the M Air T Millipore Air Tester.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. M Air T Millipore Air Tester Operation and Maintenance Instruction
   4.2. autoclave SOP
   4.3. incubator SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Always wear the appropriate personnel protective equipment (safety eye glasses and gloves).
7. **Materials:**
   7.1. M Air T Millipore Air Tester and accessories.
   7.2. M Air T Cassette pre-filled with TSA media
   7.3. autoclave
   7.4. incubator
   7.5. 70% isopropyl alcohol (IPA)
   7.6. lab towels
8. **Procedure:**
   8.1. **Using the air tester in vertical, horizontal or inclined position**
      8.1.1. When using the air tester in a vertical position, the tripod is not used.
      8.1.2. When using the air tester in a horizontal position or 30° from the horizontal position, the tripod is needed. Fix the tripod onto the air tester by screwing it into the tester fixing hole.
   8.2. **Powering up the equipment**
      8.2.1. If the equipment is used to collect samples inaccessible to power outlets, the equipment has internal rechargeable batteries ready for used. Press the ON/OFF button. LCD display will be turned on.
      Note: Make sure the battery is fully charged. The LCD will display the battery symbol if it is fully charged.

Title: M Air T Millipore Air Tester SOP

8.2.2. If the equipment is used to collect samples accessible to power outlets, plug the power adapter into an empty and convenient power outlet. LCD display will be turned on.

8.3. **Adjusting the volume to be processed**

Note: Refer to Figure 2.

8.3.1. The recommended volume is 1000 Liters.

8.3.2. Setting up volume other than the recommended volume

8.3.2.1 To access other preset volumes, press the LITERS button multiple times until you find your desired volume.

8.3.2.1 To change the volume setting, select the volume that is just below the preset volume you want to process. Then hold the LITERS button until the tester display indicates the desired sampling volume.

8.4. **Adjusting the timer**

Note: Refer to Figure 2.

8.4.1 The recommended set time is 5 minutes.

8.4.2 Setting up the time other than the recommended set time.

8.4.2.1 Hold down the START/DELAY button. The display shows preset times in increments of 5 minutes up to one hour. Select the desired time by simply releasing the START/DELAY button.

8.5 **Installing the cassette and running the tester**

Note: Refer to Figures 2, 3 and 4 as needed.

8.5.1 Define the location of the tester according to cGMP requirements

8.5.2 Ensure that pre-filled cassettes with TSA media are at room temperature before starting the test.

8.5.3 Spray down gloved hands with 70% IPA.

8.5.4 Sanitize the external surfaces of the tester with 70% IPA.

8.5.5 Position the wings of the cassette into the recessed area of the tester head.

8.5.6 Retain the cassette in position by holding on to its wings. Remove the lid and place it on the bench, internal face down.

8.5.7 Lock the micro-perforated sieve into position and remove cover

8.5.8 Press the ON/OFF button. Set the default volume and time, and then quickly press the START/DELAY button twice.
Note: The first 500 liters of volume of air collected is a slow flow rate producing a mild sound while the remaining 500 liters is a faster flow rate producing a noisy sound.

8.5.9 When the display indicates end of cycle (EOC) which is related to the set time, unlock the sieve, remove it, and put the lid back on the cassette.

8.5.10 To remove the cassette from the tester head, lift the cassette while firmly holding the edge.

8.5.11 Label (includes relevant sample data, date, initial and testing location) and incubate the cassette (37ºC for 1-3 days) in the upside down position.

8.5.12 Remove the cover from the sieve. Autoclave the sieve (without the cover) for 30 minute at 121°C.

8.5.13 Sanitize the external surfaces of the tester with alcohol.

Title: M Air T Millipore Air Tester SOP

8.5.14 Record use in the usage log.

8.6 **Evaluation of results.**

8.6.1 When incubation is complete, count the colonies on the plate.

8.6.1.1 The microbial count (CFUs) is to be stated with reference to the sample volume (i.e. CFUs/Sample Volume)

8.6.1.2 Record results in the log book.

9. Attachments:

9.1. Figure 1: Diagram of the M Air T Millipore Air Tester With Components
9.2. Figure 2: Diagram of the M Air T Millipore Air Tester With Display Buttons
9.3. Figure 3: Diagram of the M Air T Millipore Air Tester With Installed Cassette
9.4. Figure 4: Diagram of Biomanufacturing and Prep Suites

10. History:

Name	Date	Amendment
Marlo Austria	01Apr06	Initial Release
Bob O'Brien	11Sep06	Updated procedure. Changed usage log. Added Environmental Monitoring Testing Sheet.
Bob O'Brien	14Mar07	Updated date format. Updated Figures 3 and 4.

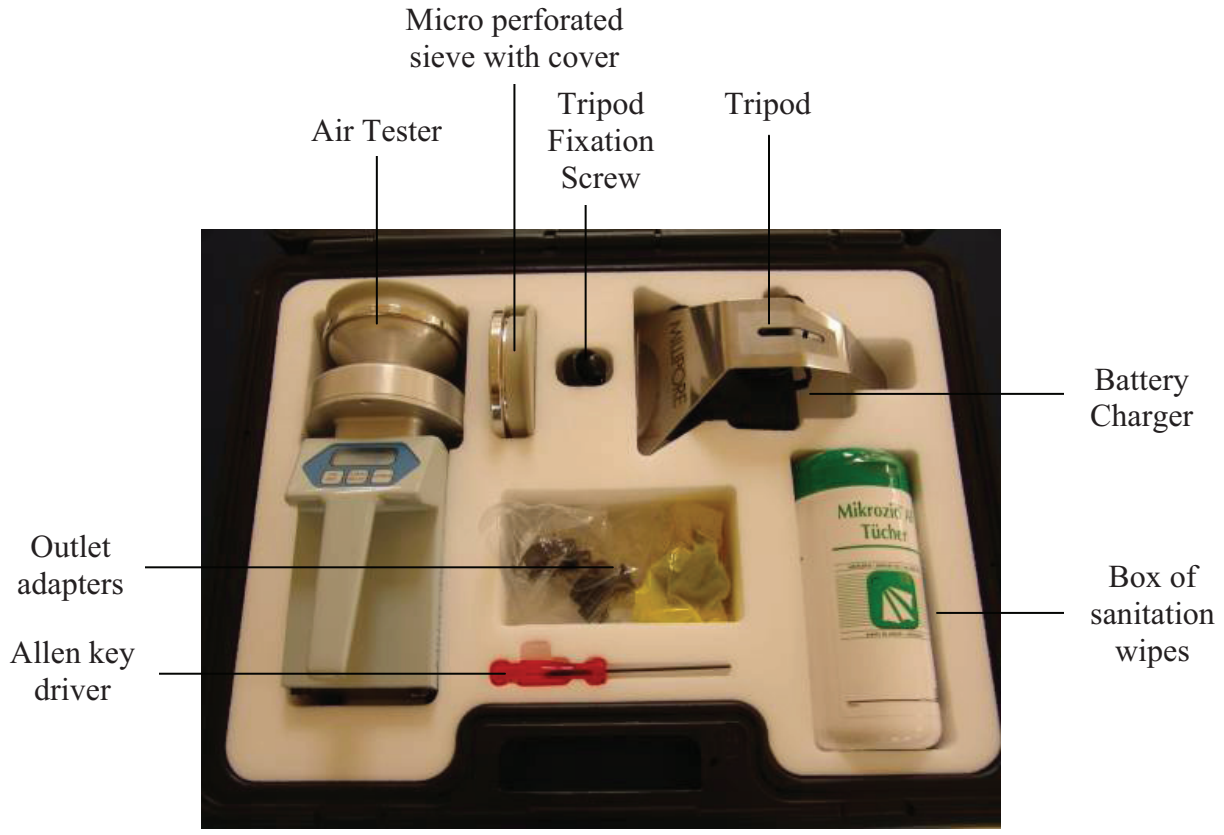


Figure 1: Diagram of the M Air T Millipore Air Tester With Components

Title: M Air T Millipore Air Tester SOP

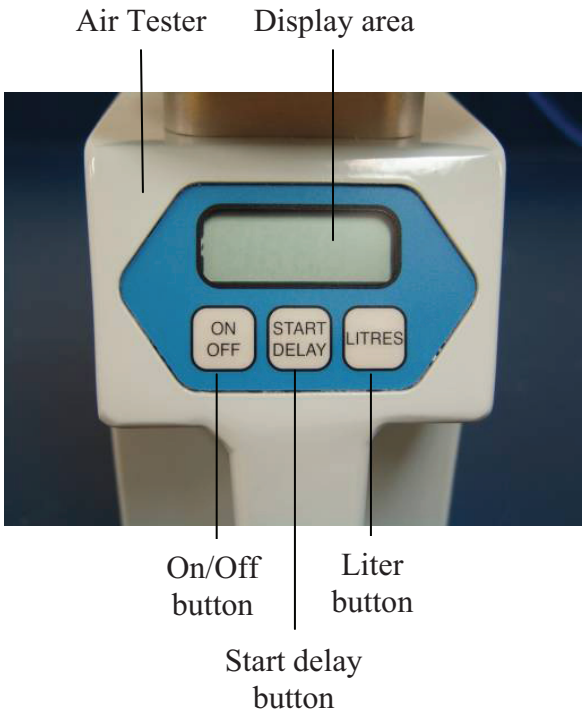


Figure 2: Diagram of the M Air T Millipore Air Tester With Display Buttons

Figure 3: Diagram of the M Air T Millipore Air Tester With Installed Cassette, Micro Perforated Sieve and Cap

Title: M Air T Millipore Air Tester SOP

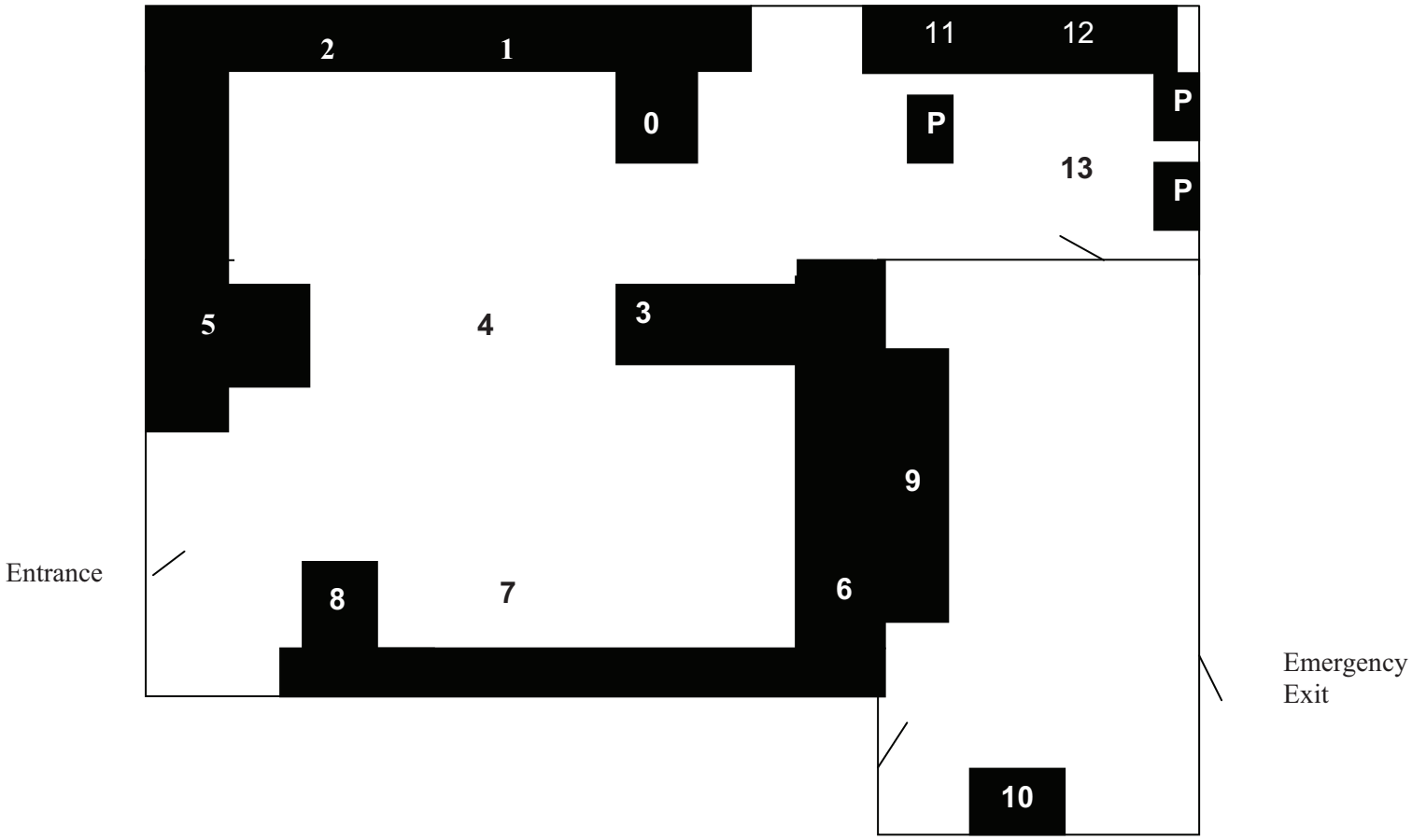


Figure 4: Diagram of Biomanufacturing and Pre Suites

Title: Gowning for Entry into Biomanufacturing Suite SOP

Approvals:
Preparer:_____Deb Audino_____ Date _____19Jun08_____
Reviewer:_____Bob O'Brien _____ Date _____19Jun08_____

1. **Purpose:**
   1.1. To describe the proper gowning procedure for personnel entering the biomanufacturing suite to minimize the number of particles and viable microorganisms in the suite.
2. **Scope:**
   2.1. Applies to gowning performed in the gowning area prior to entering the biomanufacturing suite.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Gowning for Aseptic Filling, Doxpub, Inc., Document number 02-0028-SOP-1.0
5. **Definitions:** N/A
6. **Precautions:**
   6.1. 70% isopropyl alcohol is flammable and poisonous if ingested. Avoid creating excessive mist when using spray bottles with IPA.
7. **Materials:**
   7.1. disinfecting hand soap
   7.2. sterile 70% (v/v) isopropyl alcohol (IPA)
   7.3. head cover
   7.4. hood (if needed)
   7.5. facial hair cover (if needed)
   7.6. cleanroom coverall, sterile Tyvek
   7.7. shoe covers
   7.8. non-powdered nitrile gloves
   7.9. lab tissues such as Kimwipes
8. **Procedure:**
   8.1. Employees should wear clean clothes that are not overly capable of shedding particulates (i.e., wool sweaters).
   8.2. Gowning must occur only when no one is entering or exiting the gowning area. Likewise, do not enter the gowning area while someone is gowning.
   8.3. Wearing makeup and jewelry is prohibited in the cleanrooms. If necessary, remove makeup and jewelry before proceeding to the Pre-Gowning Area.
   8.4. **Pre-Gowning Area**
      8.4.1. Enter the pre-gowning area.
      8.4.2. Sanitize hands with hand sanitizer.
      8.4.3. Sanitize safety glasses with 70% (v/v) IPA, dry with a lab tissue, and place on face.

Title: Gowning for Entry into Biomanufacturing Suite SOP

8.4.4. Don head cover. Use the mirror to verify that all hair is completely covered up to and including the hairline.
8.4.5. If applicable, don beard cover and place over facial hair. Use the mirror to verify that all hair is completely covered.
8.4.5.1. If facial hair is still exposed, replace the head cover with a hood. Verify that all hair is completely covered.
8.4.6. Disinfect hands.
8.4.7. Proceed to gowning area.

8.5. **Gowning Area**

Note: Avoid creating excess mist while using IPA throughout this procedure.

8.5.1. Put on gloves. Choose gloves that have a snug but not overly tight fit.
8.5.2. Sanitize lab bench with sterile 70% (v/v) IPA.
8.5.3. Sanitize gowning bench with sterile 70% (v/v) IPA.
8.5.4. Sanitize gloves with sterile 70% (v/v) IPA.
8.5.5. Donning the shoe covers and coverall
8.5.5.1. Obtain a gowning package.
8.5.5.1.1. Inspect the integrity of the package and verify that the package has been autoclaved.
8.5.5.2. Open the package.
8.5.5.3. Inspect the shoe covers for rips or tears.
8.5.5.3.1. If rips or tears are present, discard the shoe covers in the receptacle and repeat step 8.5.5.1.
8.5.5.4. Sit on bench and put on a single shoe cover.
8.5.5.5. Swing leg over bench to clean side of gowning area. Apply other shoe cover and stand on clean side of gowning area.
8.5.5.6. Sanitize gloves with sterile 70% (v/v) IPA.
8.5.5.7. Remove the coverall from the package.
Note: Avoid touching the coverall to the floor at all times.
8.5.5.8. Inspect the coverall for rips or tears.
8.5.5.8.1. If rips or tears are present, discard the coverall in the waste receptacle and repeat step 8.5.4.1.
8.5.5.9. Unzip the coverall.
8.5.5.10. Gather the arms and one leg of the coverall together.
8.5.5.11. Place leg in coverall and pull up the coverall. Repeat the process with the other leg. Do not allow the sleeves to touch the floor.
8.5.5.12. Pull the coverall up over the body and zip.

8.6. Sanitize gloves with sterile 70% (v/v) IPA.
8.7. After gowning, employees may enter the biomanufacturing area. While performing aseptic processing, resanitize gloves as needed. Reglove or regown if any adverse circumstances are observed or suspected that may affect the integrity of the gown components.
8.7.1. Regown by exiting the biomanufacturing suite and repeating this SOP.

8.8. **Exiting the biomanufacturing suite**

8.8.1. Proceed to the exit area.
8.8.2. Discard coverall and other cleanroom garb appropriately in the receptacle.

Title: Gowning for Entry into Biomanufacturing Suite SOP

8.8.3. Regown with fresh supplies when reentering the area.

8.9. Gowning qualifications for non-qualified personnel.

8.9.1. Non-qualified personnel (employees, outside contractors, or visitors) may not enter the biomanufacturing suite without permission from the Director of Manufactruing or QA/QC.

8.9.2. Upon permission, non-qualified personnel must gown with instructions and in the presence of a fully qualified operator and be monitored using applicable standard procedures.

8.9.3. All occurrences of non-qualified personnel entering the biomanufacturing suite between the time of room sanitization and completion of a batch must be documented using a discrepancy report.

9. Attachments: N/A

10. History:

Name	Date	Amendment
Deb Audino	22Oct05	Initial Release
Deb Audino	06Feb05	Changed donning gown from dirty side to clean side.
Deb Audino	17Jul08	College name change

Great Bay Community College
320 Corporate Drive
Portsmouth, NH 03801

Title: Four Step Gram Stain SOP

Approvals:
Preparer:___Bob O'Brien_____ Date ____04Apr08_____
Reviewer:__ Deb Audino _____ Date ____04Apr08_____

1. Purpose:
1.1. To Gram stain samples.

2. Scope:
2.1. Applies to Gram staining samples using the 3-step method to detect the presence of Gram positive and Gram negative microorganisms.

3. Responsibilities:
3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:
4.1 Gram stain pack insert
4.2. microscope SOP

5. Definitions:
5.1. Gram positive microorganism: a microorganism that stains dark purple when treated with Gram staining solutions.
5.2. Gram negative microorganism: a microorganism that stains pink when treated with Gram staining solutions.

6. Precautions:
6.1. Gram Stain reagents are harmful.Wear gloves while performing this SOP.

7. Materials:
7.1. 4-step Gram stain kit
7.2. microscope slide
7.3. P20 pipet and tips
7.4. Bunsen burner
7.5. safety gas lighter with flint
7.6. tongs
7.7. inoculation loop
7.8. isopropanol
7.9. slide staining rack
7.10. timer
7.11. water
7.12. immersion oil
7.13. microscope with 1000X magnification
7.14. lab tissues
7.15. lab towels

8. Process:
Note: Refer to Figures 1-6 as needed before performing this SOP and throughout the procedure as needed.
8.1. **Sample preparation**

Title: Four Step Gram Stain SOP

8.1.1. Label a glass microscope slide with pertinent information.
8.1.2. Prepare slide following directions for the appropriate sample source:
8.1.2.1. If sample is from a liquid culture, pipet 10µL of the culture onto the microscope slide.
8.1.2.1.1. Spread into a thin film with the pipet tip.
8.1.2.2. If sample is from a colony, pipet 10µL of water onto the slide.
8.1.2.2.1. Take a sample of the colony using a sterile loop.
8.1.2.2.2. Place the loop full of sample on the glass microscope slide, mix with water and spread into a thin film.
8.1.3. Gently heat fix the microbes to the slide.
Note: Do not overheat the slide. Excessive heating will cause atypical staining.

8.2. **Gram stain**
8.2.1. Place the slide on a slide rack to cool to room temperature before staining.
8.2.2. Cover the fixed sample on the slide with crystal violet stain and leave for approximately 1 minute.
8.2.3. Wash with a stream of water until the water runs clear.
8.2.4. Cover the fixed sample on the slide with iodine mordant and leave for approximately 1 minute.
8.2.5. Wash with a stream of water until the water runs clear.
8.2.6. Rinse with decolorizer.
8.2.7. Wash with a stream of water until the water runs clear.
8.2.8. Cover the fixed sample with safranin and leave for 30-60 seconds.
8.2.9. Wash with a stream of cold water until the water runs clear.
8.2.10. Air-dry or blot with lab tissue.
Note: Do not rub glass slide with the lab tissue.
8.2.11. View with the light microscope at 100x magnification (using oil).
8.2.12. Record whether cells are Gram positive (dark purple) or Gram negative (pink).
8.2.13. Discard the slide in the biohazard sharps container.

9. Attachments: N/A
9.1. Figure 1: Taking sample colony
9.2. Figure 2: Spreading sample colony thin film
9.3. Figure 3: Heat fix sample
9.4. Figure 4: Sample covered with crystal violet
9.5. Figure 5: Sample covered with iodine mordant
9.6. Figure 6: Sample covered with safranin

10. History:

Name	Date	Amendment
Bob O'Brien	12Jun07	Initial release
Deb Audino	04Apr08	College name change

Title: Four Step Gram Stain SOP

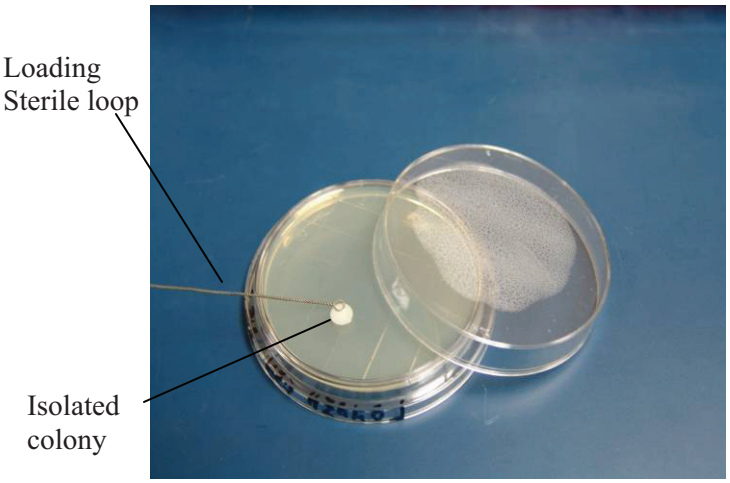


Figure 1: Taking sample colony

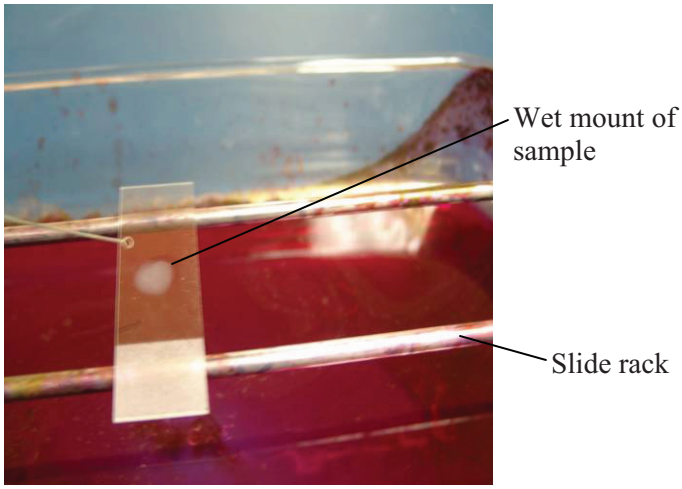


Figure 2: Spreading sample colony thin film

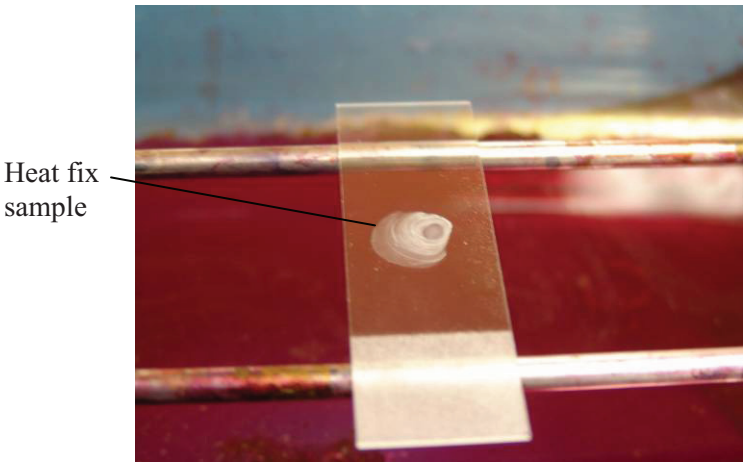


Figure 3: Heat fix sample

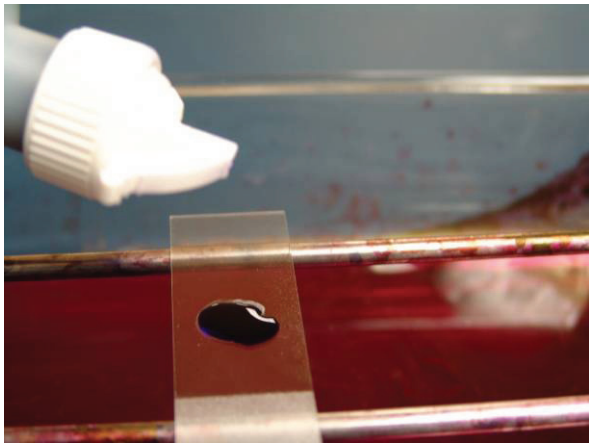


Figure 4: Sample covered with crystal violet

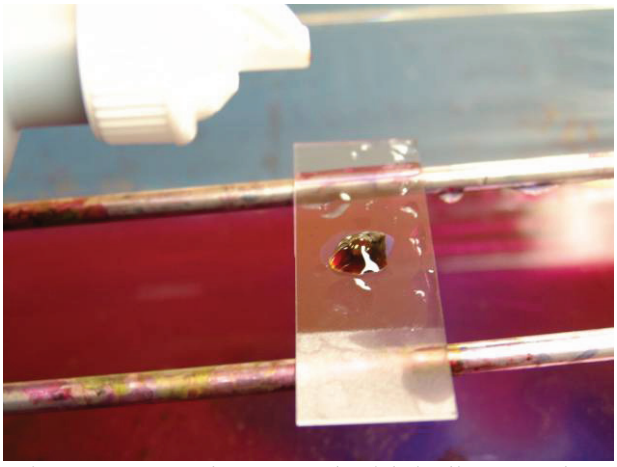


Figure 5: Sample covered with iodine mordant

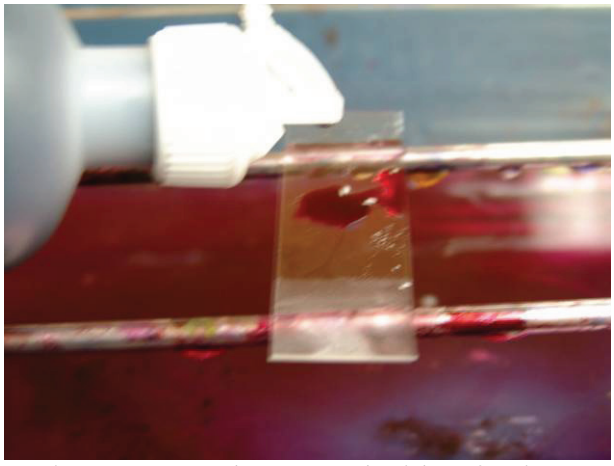


Figure 6: Sample Covered with safranin

Title: LAL ASSAY - Gel Clot Method SOP

Approvals:
Preparer:____Deb Audino_____ Date _04Apr08_____
Reviewer:___Bob O'Brien_____ Date _04Apr08_____

1. **Purpose:**
   1.1. To perform the LAL Gel Clot Assay
2. **Scope:**
   2.1. To perform the LAL Gel Clot assay on various samples such as raw materials, in process materials and the final product for determination of endotoxin concentration.
3. **Responsibility:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. LAL pack instructions
   4.2. water bath SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. LRW (LAL reagent water)
   7.2. LAL with a label sensitivity of 0.06EU/mL or 0.03EU/mL
   7.3. 5-10mL syringe and needle
   7.4. de-pyrogenated soda lime test tubes
   7.5. 100µl micropipetter and sterile pipet tips
   7.6. laboratory film, such as Parafilm
   7.7. test tube rack
   7.8. 37°C water bath
8. **Procedure:**
   8.1. **Prepare the LAL Reagent**
      8.1.1. Reconstitute the LAL by adding LRW. Swirl occasionally until completely dissolved (about 3 minutes).
   8.2. **Dilute the Sample**
      8.2.1. Set up a row of 7 de-pyrogenated test tubes and label the tubes as: Undiluted, 1:2, 1:4, 1:8, 1:16, 1:32, Negative Control.
      Note: Keep tubes covered with laboratory film when not in use.
      8.2.2. Add 100µL LRW to all tubes EXCEPT the "Undiluted" tube using the same pipet tip.
      8.2.3. Add 200µL of the sample to the "Undiluted" tube. Change pipet tip.
      8.2.4. Tip the tube so that the liquid reaches the lip of the tube and remove 100µL of the liquid. Add it to the 1:2 tube.
      8.2.5. Vortex the tube for 4 seconds. Change pipet tip.

Title: LAL ASSAY - Gel Clot Method SOP

8.2.6. Tip the tube so that the liquid reaches the lip of the tube and remove 100µL of the liquid. Add it to the 1:4 tube.
8.2.7. Vortex mix the 1:4 tube for 4 seconds. Change pipet tip.
8.2.8. Tip the tube so that the liquid reaches the lip of the tube and remove 100µL of the liquid. Add it to the 1:8 tube.
8.2.9. Vortex mix the 1:8 tube for 4 seconds. Change pipet tip.
8.2.10. Tip the tube so that the liquid reaches the lip of the tube and remove 100µL of the liquid. Add it to the 1:16 tube.
8.2.11. Vortex mix the 1:16 tube for 4 seconds. Change pipet tip.
8.2.12. Tip the tube so that the liquid reaches the lip of the tube and remove 100µL of the liquid. Add it to the 1:32 tube.
8.2.13. Vortex mix the 1:32 tube for 4 seconds. Change pipet tip.
8.2.14. Tip the 1:32 tube, remove 100µL and DISCARD it.
8.2.15. Do not add sample to the negative control tube.
Note: All the tubes should have 100µL of liquid.

8.3. **Add the LAL Reagent**

8.3.1. Starting with the negative controls and proceeding from the lowest to the highest sample concentration, add 100µL LAL to each tube. Tips need to be changed after each addition.
Note: LAL must be added to all tubes within 2 minutes.
8.3.2. Shake the test tube rack vigorously for 30 seconds to mix the LAL and sample.

8.4. **Incubate the Tubes**

8.4.1. Cover the tubes with laboratory film and CAREFULLY place the rack in the water bath at about 37°C (Do not disturb other racks). Record the temperature and time.
Note: Do not disturb the tubes during the incubation. Once a clot is broken, it will not re-form.
8.4.2. Incubate for approximately 60 minutes.

8.5. **Analyze the Tubes**

8.5.1. Remove the tubes one at a time from the incubator and invert them SLOWLY and SMOOTHLY. Score tubes as positive if a firm clot has formed. Score tubes as negative if a gel holds, but collapses after the tube is fully inverted.
8.5.2. Record data.
8.5.3. Determine the amount of endotoxin in the samples using the formula:
Endotoxin concentration < LAL label sensitivity x dilution factor of most concentrated sample NOT to clot.

9. Attachments:

9.1. Data Table

Title: LAL ASSAY - Gel Clot Method SOP

10. History:

Name	Date	Amendment
Deb Audino	2001	Initial Release
Deb Audino	2003	Added more detailed directions.
Deb Audino	02Feb05	Replaced CSE with a sample.
Deb Audino	10Oct05	Added undiluted sample, data table, and how to calculate endotoxin level.
Deb Audino	04Apr08	College name change

	Undiluted 1	1:2	1:4	1:8	1:16	1:32	Negative Control
Sample ID _____							
Sample ID _____							

Title: LAL Assay – Cambrex QCL-1000® Endpoint Colorimetric Microplate Method SOP

Approvals:
Preparer:____Sheila Byrne_____ Date _10Jul06_____
Reviewer:___Linda Rehfuss_____ Date _10Jul06_____

1. **Purpose:**
   To perform a LAL colorimetric endpoint assay for the detection of endotoxins.
2. **Scope:**
   This procedure is intended as an endotoxin test for human and animal parenteral drugs, biological products and medical devices.
3. **Responsibilities:**
   3.1 It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2 It is the responsibility of the students to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1 Cambrex LAL QCL-1000 Brochure
   4.2 Biotek Automated Microplate Reader SOP
   4.3 Biotek Automated Microplate Reader Model ELX808 Operators Manual
   4.4 Biotek KC Junior User's Guide
5. **Definitions:**
   5.1 Endotoxin: the lipopolysaccaride component of the cell wall of Gram negative bacteria
6. **Precautions**:
   6.1 Endotoxin Standard contains human source material. Exercise caution when handling.
7. **Materials:**
   7.1 Limulus Amebocyte Lysate (LAL) (*Cambrex*)
   7.2 Endotoxin Standard (*Cambrex*)
   7.3 Chromogenic Substrate (*Cambrex*)
   7.4 LAL Reagent Water
   7.5 Stop Reagent (e.g. 25% v/v glacial acetic acid in water)
   7.6 endotoxin-free glass dilution tubes
   7.7 microplates (endotoxin-free, disposable with lid)
   7.8 pipettors with sterile tips
   7.9 10 ml Pipettes (individually wrapped, sterile)
   7.10 vortex mixer
   7.11 dry bath heater with microplate adapter
   7.12 microplate Reader
   7.13 stopwatch or timer
   7.14 Parafilm®

Title: LAL Assay – Cambrex QCL-1000® Endpoint Colorimetric Microplate Method SOP

7.15 0.1N NaOH (sodium hydroxide) and 0.1N HCl (hydrochloric acid) dissolved in LAL reagent water for pH adjustment of sample if necessary

8. Procedure

8.1 **Specimen collection and preparation:**

8.1.1 Specimens should be collected aseptically in non-pyrogenic containers.

8.1.2 All materials coming in contact with the specimen or reagents must be endotoxin-free.

8.1.3 Specimens may be stored at 2-8°C for less than 24 hours. Specimens stored for longer than 24 hours should be frozen.

8.1.4 It may be necessary to adjust the sample to within the pH range of 6.0 to 8.0 using endotoxin-free 0.1 N NaOH or 0.1 N HCl. Always measure an aliquot of the sample to avoid contamination from the pH electrode.

8.2 **Reagent Preparation:**

8.2.1 Prepare the stop reagent.

8.2.1.1 Prepare sufficient 25% vol./vol. glacial acetic acid in distilled water to allow delivery of 50 µl per well.

8.2.2 Prepare the LAL.

8.2.2.1 Reconstitute the LAL by adding 1.4 ml of LAL Reagent Water. Swirl gently to avoid foaming. If more than one vial is needed, pool before use. Use promptly or freeze at -10°C. for up to 1 week. Thaw and use only once.

8.2.3 Prepare the Chromogenic Substrate.

8.2.3.1 Reconstitute the Chromogenic Substrate by adding 6.5 ml of LAL Reagent Water. May be stored at 2-8°C up to four weeks. Protect from long-term exposure to light.

8.2.4 Prepare the Endotoxin Stock.

8.2.4.1 Reconstitute the Endotoxin by adding 1.0 ml of LAL Reagent Water warmed to room temperature. Vortex vigorously for at least 15 minutes. May be stored at 2-8°C up to four weeks. Endotoxin must be warmed to room temperature and vortexed vigorously before each use.

8.2.5 Prepare the standard curve (**Table 1**).

8.2.5.1 Prepare a 1.0 E.U. /ml standard.

8.2.5.1.1 In an endotoxin- free glass tube dilute 0.1ml of the endotoxin stock solution with [(X - 1) ÷ 10] ml of LAL Reagent Water where X= the concentration of the vial of stock endotoxin which varies by kit. (See Certificate of Analysis for concentration of stock endotoxin.) For example, if X = 23 E.U. /ml. then dilute 0.1 ml of endotoxin stock solution with 2.2 ml [(23 -1) ÷ 10] of LAL Reagent Water. Vortex vigorously for 1 minute. If using Parafilm, the side facing the paper may be considered endotoxin-free.

8.2.6 Prepare a 0.5 E.U. /ml standard.

8.2.6.1 In an endotoxin-free glass tube dilute 0.5 ml of the 1.0 E.U. /ml

Title: LAL Assay – Cambrex QCL-1000® Endpoint Colorimetric Microplate Method SOP

standard with 0.5 ml of LAL Reagent Water. Vortex vigorously for 1 minute.

8.2.7 Prepare a 0.25 E.U./ml standard.

8.2.7.1 In an endotoxin-free glass tube dilute 0.5 ml of the 1.0 E.U. /ml standard with 1.5 ml of LAL Reagent water. Vortex vigorously for 1 minute.

8.2.8 Prepare a 0.1 E.U. /ml standard.

8.2.8.1 In an endotoxin-free glass tube dilute 0.1 ml of the 1.0 E.U./ml standard with 0.9 ml of LAL Reagent Water. Vortex vigorously for 1 minute.

Table 1

Final Endotoxin Concentration EU/ml	Endotoxin Stock Solution	Endotoxin Std. Solution 1 EU/ml	LAL Reagent Water
1.0	0.1 ml		(X-1) ÷10 ml
0.5		0.5 ml	0.5 ml
0.25		0.5 ml	1.5 ml
0.1		0.1 ml	0.9 ml

8.3 **Test Procedure (Table 2):**

8.3.1 Pre-equilibrate the microplate at 37± 1°C in the heating adapter block.

8.3.2 Pre-warm a sufficient aliquot of Chromogenic Substrate to 37± 1°C.

8.3.3 While leaving the microplate at 37°C carefully dispense 50 µl of standard, sample or LAL Reagent Water into the appropriate microplate well. Each series of determinations must include a blank (LAL Reagent Water) plus 4 endotoxin standards run in duplicate.

8.3.4 At T = 0, add 50 µl of LAL to the first microplate well or first column of wells if using a multichannel pipettor. Begin timing as the LAL is added. It is important to be consistent in the order of reagent addition to wells and in the rate of pipetting. Dispense to all wells being used.

8.3.5 Gently mix the microplate with a back and forth motion while it is still in the heat block.

8.3.6 At T = 10 minutes add 100 µl of Chromogenic Substrate (prewarmed to 37 ± 1°C). Pipette to the wells in the same order and at the same rate as step 8.2.4.

8.3.7 Gently mix the microplate with a back and forth motion while it is still in the heat block.

8.3.8 At T = 16 minutes add 50 µl of stop reagent. Pipette to the wells in the same order and at the same rate as in the previous steps.

8.3.9 Remove the plate from the heat block and gently tap repeatedly or swirl to mix.

Title: LAL Assay – Cambrex QCL-1000® Endpoint Colorimetric Microplate Method SOP

8.3.10 Read the absorbance of each well at 405 nm on the plate reader according to its SOP. Remove lid from plate when reading.

Table 2

	Sample or Standard	Blank
Test sample or standard at 20-25°C	50 µl	
LAL Reagent Water		50 µl
LAL Lysate	50 µl	50 µl
Mix and incubate at 37±°C	10 min.	10 min.
Chromogenic Substrate at 37±1°C	100 µl	100 µl
Mix and incubate at 37±1°C	6 min.	6 min.
Stop Reagent	50 µl	50 µl
Mix		

8.4 **Calculation of Endotoxin Concentration**

Note: Plate reader software may be used to perform the following calculations.

8.4.1 Subtract the mean absorbance of the blank from the mean absorbance of the standards and samples to calculate mean Δ absorbance.

8.4.2 Graph by plotting the mean Δ absorbance for the four standards on the y-axis vs. the corresponding endotoxin concentration in EU/ml on the x-axis and drawing a best fit straight line between the points.

8.4.3 Use graph to determine endotoxin values of samples. The absorbance is linear in the concentration range of 0.1 to 1.0 EU/ ml of endotoxin. If the concentration of the sample is greater than 1.0 EU/ml, dilute the sample 5-fold in LAL Reagent Water and retest. Calculate the concentration of the diluted sample and multiply by 5 to determine the original endotoxin concentration.

9. Attachments: N/A

10. History:

Name	Date	Amendment
Shelia Byrne	10Jul06	Initial Release

Title: Bio-Tek Elx808UI Automated Microplate Reader Standalone SOP

Approvals:
Preparer:___Kari Britt_____ Date ____10Aug10_____
Reviewer:__Sonia Wallman_____ Date ____10Aug10_____

1. **Purpose:**
   1.1. Operation of the Bio-Tek Elx808UI Automated Microplate Reader.
2. **Scope:**
   2.1. Applies to the Bio-Tek Elx808UI Automated Microplate Reader for performing optical density testing on solutions.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensre that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Bio-Tek Automated Plate Reader Operators Manual
5. **Definitions:**N/A
6. **Precautions:**N/A
7. **Materials:**
   7.1. samples, standards and controls to be tested
   7.2. micropipettor.
   7.3. 96-well microplate (U, V, or flat-bottom wells are acceptable).
8. **Procedure:**
   8.1. **Preparation**
      8.1.1. Assemble samples to be tested . A minimum of 100µL for each well is required.
      8.1.2. Load samples into microplate starting at the top left corner (location A1). Load proceeding samples down the microplate, B1, C1, D1, etc. Refer to Figure 1 for a map of the microplate.
   8.2. **Operation**
      8.2.1. Turn the power switch to the ON position (located on the rear of the right side panel). The equipment will perform a system self-test to verify that the components are operating properly and that the internal software has not been corrupted (less than one minute).
      8.2.2. Press the **READ** key on the bottom right corner of the control pad or press the corresponding softkey for **READ** below the LCD display. See Figure 2.
      8.2.3. Type in "01" to select assay number 01, Quick Read assay (if not already selected). Press **Enter** to continue.
      8.2.4. Select either **Single** or **Dual** wavelength and press Enter.
      Note: If Dual wavelength is selected, the previous wavelength setting for Single will be used for the measuring wavelength. The second wavelength will be the reference wavelength.
      8.2.5. Press the softkey corresponding to the wavelength to be used. Press **Enter.**

Title: Bio-Tek Elx808UI Automated Microplate Reader Standalone SOP

8.2.6. Type in the number of samples to be tested. The samples will be measured starting with well A1, then B1, C1, etc. If more than 8 samples were loaded, the machine will automatically move to column 2 and begin measuring at well A2.
8.2.7. Press **Enter** to continue.
8.2.8. Open the lid to the carrier and load microplate into reader. Well A1 must be located in the top left corner. See Figure 3.
8.2.9. Close the cover and press the **READ** key on the control pad.
8.2.10. When the reader has completed the measurements, data will automatically be sent to printer.
8.2.11. When finished, turn the power off.

9. Attachments:

9.1. Figure 1: Microplate Map
9.2. Figure 2: Control Pad
9.3. Figure 3: Loading a Microplate

10. History:

Name	Date	Amendment
Ellery Raitt	03Mar05	Initial release
Kari Britt	08Aug10	College name change. Updated date format. Also, made formatting edits as needed throughout.

Title: Bio-Tek Elx808UI Automated Microplate Reader Standalone SOP

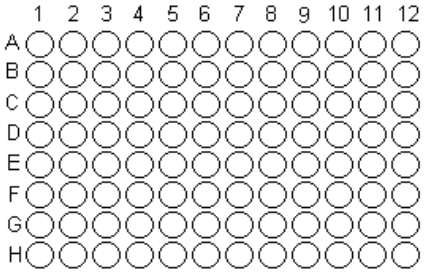


Figure 1: Microplate Map

Figure 2: Control Pad

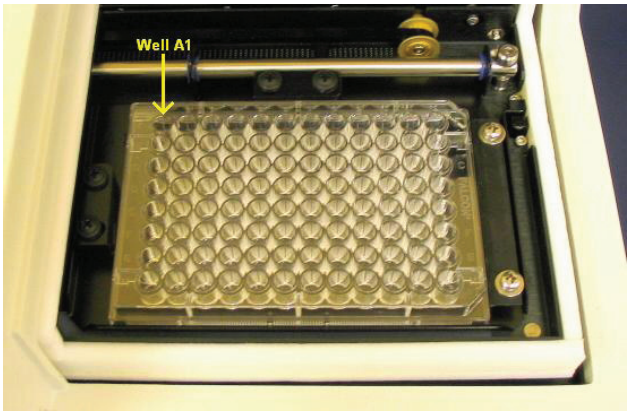


Figure 3: Loading a Microplate

Title: Mycoplasma Testing SOP

Approvals:
Preparer:___Deb Audino_____ Date ___04Apr08_____
Reviewer:__Bob O'Brien_____ Date ___04Apr08_____

1. **Purpose:**
   1.1. Testing of samples for presence of mycoplasma.
2. **Scope:**
   2.1. Applies to testing of solutions including media and cultures for presence of mycoplasma.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Takara mycoplasm testing protocol
   4.2. Amersham Bioscience puReTaq Ready to go PCR beads protocol
   4.3. thermocycler SOP
   4.4. gel documentation System SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Ethidium Bromide is a mutagen. Use care and wear double gloves.
   6.2. UV light can damage eyes, wear UV shields when using the UV light box
7. **Materials:**
   7.1. Takara Mycoplasma Detection PCR Kit (Catalog number: 6601)
      7.1.1. Takara forward primer diluted 1:5 with PCR grade water
      7.1.2. Takara reverse primer diluted 1:5 with PCR grade water
      7.1.3. Takara control template diluted 1:5 with PCR grade water
   7.2. Amersham Bioscience pure Taq Ready to Go PCR beads (Catalog number: 27-9557-01)
   7.3. PCR grade water
   7.4. mineral oil
   7.5. agarose
   7.6. 10x TBE buffer
   7.7. 1% ethidium bromide solution
   7.8. DNA sample buffer
   7.9. 1kb DNA ladder (New England Biolabs, Catalog number: 3232L)
   7.10. sterile filter pipet tips (0-30µL and up to 200µL)
   7.11. thermal cycler
   7.12. horizontal electrophoresis box
   7.13. power Supply
   7.14. gel documentation system with UV light
8. **Procedure:**
   8.1. **PCR amplification**
      8.1.1. **Preparation of samples**

Title: Mycoplasma Testing SOP

8.1.1.1. Verify that a bead is visible at the bottom of each tube. If necessary tap the tube against a hard surface to force the bead to the bottom of the tube.

8.1.1.2. Label tubes as: sample, +C1, +C2, -C
Write the date and your initials on all tubes.

8.1.1.3. Add the following to each tube containing a PCR bead:

	Sample	+ Control **1**	+ Control **2**	- Control
Sterile distilled water	18µL	22µL	17µL	23µL
Forward primer	1µL	1µL	1µL	1µL
Reverse primer	1µL	1µL	1µL	1µL
Control DNA	n/a	1µL	1µL	n/a
Sample DNA	5µL	n/a	5µL	n/a

8.1.1.4. Mix the contents of the tubes by gently pipetting up and down.

8.1.1.5. Overlay each reaction mix with 50µL mineral oil.

8.1.2. **Amplification of samples**

8.1.2.1. Turn on instrument.

8.1.2.2. Verify wells in the themal cycler have mineral oil and add mineral oil if needed.

8.1.2.3. Verify that PCR program number 47 has not been changed.
Program should be:

94C	5min	1 cycle
94C	1min	\
55C	2min	35 cycles
72C	1min	/
4C	10min	

If necessary, edit the program.

8.1.2.4. Place all tubes in the thermal cycler.

8.1.2.5. Start program number 47.

8.1.2.6. When program is complete, shut off instrument and remove tubes.

8.1.2.7. Store tubes at -20°C.

8.2. **Analysis by Electrophoresis**

8.2.1. **Preparation of 1x TBE Running Buffer**

8.2.1.1. Dilute the 10x TBE to 1x TBE by combining 100mL 10x TBE with 900mL DI water. Mix well.

8.2.2. **Preparation of 2% Agarose Gel**

8.2.2.1. Assemble gel box WITHOUT the comb.

8.2.2.2. Weigh out 2 ±0.1 grams of agarose and place into a 250mL Erlenmyer flask.

8.2.2.3. Add 100mL 1xTBE buffer to the agarose.

8.2.2.4. Microwave the agarose mixture until it begins to boil (~3 minutes).

8.2.2.5. Carefully remove from microwave and swirl to mix.

8.2.2.6. Pour agarose mix into the gel box tray until the height of the agarose mix is approximately 0.5cm.

Title: Mycoplasma Testing SOP

8.2.2.7. Add 10µL ethidium bromide and mix with the pipet tip.
Reminder: Ethidium bromide is a mutagen, use care when handling and wear double gloves.
8.2.2.8. Place the comb into the tray.
8.2.2.9. Allow the gel to set (approximately 30 minutes).
8.2.2.10. When the gel is set, remove the comb, reverse the tray and add enough 1X TBE buffer to just cover the gel.

8.2.3. **Running the Gel**

8.2.3.1. Remove PCR tubes from the -20°C freezer and IMMEDIATELY remove as much mineral oil from the top as soon as possible before the samples thaw.
8.2.3.2. Add 10µL sample buffer to each tube and mix well.
8.2.3.3. Load 15µL of each sample into individual wells. Record position of samples.
8.2.3.4. Load 10µL of DNA ladder into an adjacent well. Record position of ladder.
8.2.3.5. Connect the lid to the electrophoresis apparatus.
8.2.3.6. Connect the leads to the power supply making sure to use the color codes.
8.2.3.7. Turn on the power supply and run at 100Vfor approximately 1-2 hours.
8.2.3.8. Turn off the power supply.

8.2.4. **Analysis**

8.2.4.1. Wearing double gloves, carefully remove gel and tray from the apparatus and place into a storage container designated for ethidium bromide.
8.2.4.2. Observe under the UV light box.
Reminder: Use the UV shield.
8.2.4.3. Take a photograph of the gel.
8.2.4.4. Molecular weight of the + Control PCR band should be 810bp.

9. Attachments:

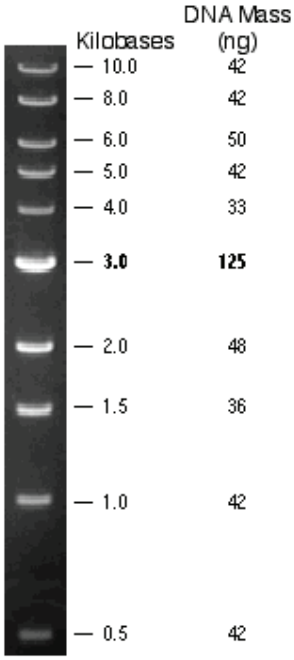
9.1. Data Table
9.2. Picture of 1kb DNA Ladder

10. History:

Name	Date	Amendment
Deb Audino	02Feb05	Initial Release
Deb Audino	10Oct05	Added catalog number for the Takara kit. Added concentration of ethidium bromide stock solution. Reduced volume of ethidium bromide solution for gel. Added attachments.
Deb Audino	04Apr08	College name change

Title: Mycoplasma Testing SOP

Lane Position	ID of PCR reaction loaded	Presence (+) or Absence (-) of 810bp band
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		



1kb DNA Ladder (New England Biolabs)

Quality Control Technician (Microbiology)

REF	Key Functions & Tasks (Quality Control Microbiology Technician)
1	**Work in compliance with EH&S.**
1.a	Wear appropriate personal protective equipment.
1.b	Work in controlled environments.
1.c	Participate in emergency drills and emergency response teams.
1.d	Use routine lab safety procedures, identify unsafe conditions, and take corrective action.
1.e	Appropriately and safely access production and laboratory equipment.
1.f	Handle, label, and dispose of hazardous / biohazard materials.
1.g	Access and utilize MSDS.
1.h	Perform permitting procedures.
1.i	Carries out operations with attention to OSHA and EPA regulations, and other applicable state and federal regulations.
1.j	Participate in all company safety training and audits as required.
1.k	Assists with waste treatment operations.
1.l	Dispose of wastes .
1.m	Follows SOPs, written test procedures, safety, regulatory requirements, and approved license requirements.
1.n	Use hoods where appropriate.
1.o	Dispose of sharps properly.
2	**Work in compliance with cGMPs.**
2.a	Follow SOPs for all operations.
2.b	Records laboratory data and completes lab records as required.
2.c	Maintain equipment and instrument logbooks.
2.d	Control and receipt of raw materials.
2.e	Maintain training documentation.
2.f	Maintain equipment and methods in a validated state.
2.g	Working in controlled/classified areas (gowning, aseptic technique).
2.h	Ensure appropriate flow of personnel, equipment, and materials.
2.i	Change control for process, equipment, and documentation.
2.j	Label and apply status to equipment and materials.
2.k	Identify and report exception events and CAPA.
2.l	Review data.
2.m	Participate in change control activities.
2.n	Archive documentation/data.
3	**Clean and maintain production areas.**
3.a	Housekeeping / pest control.
3.b	Sanitize and clean of controlled spaces.
3.c	Preparation of cleaning materials and solutions
3.d	Document cleaning.
4	**Maintain effective communication.**
4.a	Maintain security and confidentiality.
4.b	Respond appropriately to internal auditors and external inspectors.
4.c	Assist in writing, reviewing, and commenting on technical documents.
4.d	Consult appropriately with internal customers.
4.e	Report result to appropriate departments.
4.f	Interact with vendors.

REF	Key Functions & Tasks (Quality Control Microbiology Technician)
5	**Receive and/or collect samples.**
5.a	Receive/collect samples (stability, in-process, water, raw materials, final product, environment, validation) per batch records or plans.
5.b	Monitor controlled equipment.
5.c	Maintain equipment logs.
5.d	Label samples appropriately.
5.e	Record sample collection and distribution (storage and chain of custody).
6	**Laboratory Work.**
6.a	Prepare reagents.
6.b	Prepare samples.
6.c	Review testing procedures
6.d	Execute testing.
6.e	Perform mathematical and statistical calculations as appropriate.
6.f	Evaluate data with regard to specification.
6.g	Perform scheduled sanitizations of hoods.
6.h	Prepare and standardize probes and ancillary instruments.
6.i	Visually inspect equipment.
6.j	Maintain equipment logs and status tags.
6.k	Complete, review and approve equipment process records.
7	**Perform Microbiological Testing and Culturing.**
7.a	Take environmental monitoring samples from production areas and personnel.
7.b	Perform microbial identification.
7.c	Test for mycoplasma and virus.
7.d	Test for endotoxin/pyrogen using LAL (e.g., water, in-process, final product)
7.e	Test for bioburden (e.g., water, in-process, final product)
7.f	Test sterilization cycles with biological indicators.
7.g	Test for quality of media/reagents using growth promotion tests.
7.h	Conducts sterility testing on in-process materials and finished products.
7.i	Qualify sanitizing agents and disinfectants.
7.j	Maintain reference cultures.
8	**Managing Information**
8.a	Support data trending activities.
8.b	Utilize Laboratory Information Management Systems (LIMS)
8.c	Records observations, generates reports, and maintains accurate records
9	**Provide Technical and/or Validation Support**
9.a	Troubleshoots basic technical issues and investigations of OOS results, instrument malfunctions,and methodology problems.
9.b	Assist in executing validation procedures
10	**Maintain laboratory systems and equipment**
10.a	Perform preventive maintenance (PM).
10.b	Standardize laboratory instrumentation.
10.c	Identify and assist in troubleshooting instrument and equipment failures.

Upstream - GFP

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Title: Batch Culture of *Escherichia coli* Recombinant for Jellyfish Green Fluorescent Protein SOP

Approvals:
Preparer:_____Deb Audino _____ Date _____18Apr07_____
Preparer:_____Kari Britt _____ Date _____18Apr07_____

1. **Purpose:**
   1.1. To produce a batch culture of bacterial host cells.
2. **Scope:**
   2.1. Applies to the production of green fluorescent protein from recombinant *E. coli* cells.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. LB Broth manufacturer instructions
   4.2. LB Agar manufacturer instructions
   4.3. autoclave SOP
   4.4. shaking incubator SOP
   4.5. water bath SOP
   4.6. spectrophotometer SOP
   4.7. incubator SOP
   4.8. pH meter SOP
   4.9. Gram stain SOP
   4.10. microscope SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Recombinant *E. coli* is a BL2 microorganism. Use BL2 safety measures and discard waste in biohazard containers.
7. **Materials:**
   7.1. 1mL vials of *E.coli* recombinant for GFP (-86°C freezer)
   7.2. Luria-Bertani (LB) Broth premixed powder (room temp)
   7.3. Luria-Bertani (LB) Agar premixed powder (room temp)
   7.4. Arabinose (room temp)
   7.5. Ampicillin powder (4-8°C)
   7.6. 70% Isopropanol (room temp)
   7.7. deionized water
   7.8. small beaker
   7.9. 30cc syringe
   7.10. sterile syringe filter (0.2µm)
   7.11. sterile 50mL centrifuge tube
   7.12. 500 mL Erlenmeyer shake flask with cap
   7.13. 2L Erlenmeyer flask with cap
   7.14. petri dishes (100x15mm, approx. 55 per batch of LB agar)

Title: Batch Culture of *Escherichia coli* Recombinant for Jellyfish Green Fluorescent Protein SOP

7.15. 125 mL glass bottle with cap
7.16. 25mL, 100mL, 250mL, 1000mL graduated cylinders
7.17. magnetic stir plate
7.18. magentic stir bars
7.19. autoclave tape
7.20. laboratory film such as Parafilm
7.21. sterile pipettes (25mL, 2mL) and pipet pumps
7.22. 1.5mL microfuge tubes
7.23. cell Spreader
7.24. spectrophotometer Cuvettes
7.25. balance
7.26. autoclave
7.27. 55°C water bath
7.28. 37°C radial shaking incubator
7.29. spectrophotometer
7.30. micropipettors and sterile pipette tips
7.31. micro-scale pH meter (pH7 and pH4 commercially prepared buffers)
7.32. microscope with 1000x magnification
7.33. Gram stain reagents

8. Procedure:

8.1. **Solution and Media Preparation**

8.1.1. **Culture Broth:** Luria-Bertani (LB) Broth, Ampicillin (0.09mg/mL), Arabinose (1.8mg/mL)

8.1.1.1. Gather the following items and place on a clean lab bench area:
500mL Erlenmeyer shake flask with cap
125mL glass bottle with cap
250mL graduated cylinder
magnetic stir bar and magnetic stir plate
autoclave tape

8.1.1.2. Weigh out approximately:

LB Broth premix	4g
arabinose	0.36g

8.1.1.3. Add LB broth premix and arabinose to a clean 500mL shake flask.
8.1.1.4. Measure about 200mL of deionized water using a 250mL graduated cylinder and add to the LB/ARA broth.
8.1.1.5. Stir to dissolve the ingredients using a magnetic stir bar and stir plate.
8.1.1.6. Measure approximately 100mL of the LB/ARA broth using the 250mL graduated cylinder and transfer to the 125mL glass bottle.
8.1.1.7. Remove the stir bar.
8.1.1.8. Place caps on 500mL shake flask and 125mL bottle loosely to allow air flow (but not so cap can fall off). Place a small piece of autoclave tape on each.
8.1.1.9. Label shake flask: LB/ARA, [date], [initials], GFP, [group #].
8.1.1.10. Label glass bottle: LB/ARA, [date], [initials], GFP, [group #].

8.1.2. Autoclave the broth per the following instructions.

8.1.2.1. Place the following items into the autoclave:

Title: Batch Culture of *Escherichia coli* Recombinant for Jellyfish Green Fluorescent Protein SOP

500mL shake flask of LB/ARA medium
125mL glass bottle of LB/ARA broth only

8.1.2.2. Autoclave per SOP for 20 minutes at 121°C.
8.1.2.3. Remove items from the autoclave when pressure reaches <5 psi and <80°C.
8.1.2.4. Tighten cap on 125mL glass bottle and shake flask. Store at room temperature.

8.1.3. **Culture Agar:** Luria-Bertani (LB) Agar, Ampicillin (0.09mg/mL), Arabinose (1.8mg/mL).
8.1.3.1. Gather the following on a clean lab bench area:
Clean 2L Erlenmeyer flask with cap
Clean 1L graduated cylinder
Magnetic stir bar and magnetic stir plate
8.1.3.2. Weigh out approximately:
LB Agar (premix) 35g
Arabinose 1.8g
8.1.3.3. Add ingredients to a clean 2L flask.
8.1.3.4. Add about 1L of deionized water.
8.1.3.5. Stir to mix using a magnetic stir bar and stir plate.
Note: The agar may not dissolve completely until it is autoclaved.
8.1.3.6. Remove the stir bar.
8.1.3.7. Place cap on 2L Erlenmeyer flask loosely to allow air flow (but not so cap can fall off). Place a small piece of autoclave tape on flask and bottle caps.
8.1.3.8. Label flask: LB/ARA, [date], [initials].
8.1.3.9. Autoclave for 20 minutes at 121 °C.
8.1.3.10. Remove from autoclave when pressure reaches <5 psi and <80°C.
8.1.3.11. Cool to the touch in a 55°C water bath.

8.1.4. **Ampicillin Stock Solution** (6mg/mL)
8.1.4.1. Gather the following items on clean lab bench area:
Small beaker
25mL graduated cylinder
Sterile syringe filter (0.2µm) and 30cc syringe
Sterile 50mL centrifuge tube
8.1.4.2. Weigh out approximately 0.12g of ampicillin powder, place in small beaker.
8.1.4.3. Measure approx. 20mL deionized water using the 25mL graduated cylinder. Add to the ampicillin and swirl to dissolve.
8.1.4.4. Sterile filter solution using sterile syringe filter:
8.1.4.4.1. Draw up solution into 30cc syringe.
8.1.4.4.2. Attach sterile syringe filter (clear side of filter faces syringe).
8.1.4.4.3. Depress plunger and expel solution into sterile 50mL centrifuge tube.
8.1.4.5. Label tube: AMP 6mg/mL, [date], [initials], storage: -20°C, disposal: autoclave/then drain.

8.1.5. **Add Ampicillin**
8.1.5.1. Gather the following on a clean lab bench area:

Title: Batch Culture of *Escherichia coli* Recombinant for Jellyfish Green Fluorescent Protein SOP

Ampicillin solution (6mg/mL)
Cooled media
2mL and 25mL pipets and pump

8.1.5.2. Aseptically add 1.5mL of sterile filtered ampicillin stock solution (6mg/mL) to shake flask containing 100mL culture medium (media must be cool to the touch!), swirl to mix.

8.1.5.3. Revise the label on the shake flask to reflect that Ampicillin has been added (LB/ARA/AMP).

8.1.5.4. Aseptically add 15mL of sterile filtered ampicillin stock solution (6mg/mL) to the 2L flask containing 1L agar medium, swirl to mix.

8.1.5.5. Revise the label on the 2L flask to reflect that Ampicillin has been added (LB/ARA/AMP).

8.1.6. **Pour Culture Plates**

8.1.6.1. Disinfect a lab bench by spraying with 70% isopropanol.

8.1.6.2. Spread 55 sterile plastic petri dishes on the disinfected lab bench (save plastic sleeve).

8.1.6.3. Label plates on the edge of the bottom plate (as small as possible) with description of media, LB/AMP/ARA, the date and operator initials.

8.1.6.4. Pour agar plates and allow agar to solidify undisturbed with covers on.

8.1.6.5. Turn over plates and incubate plates 24-48 hours, inverted at 37ºC.

8.1.6.6. Check for contamination, autoclave and discard any plates showing microbial growth.

8.1.6.7. Store proofed plates, stacked upside down in their plastic sleeve, at room temperature.

8.1.7. **Proof the LB Broth**

8.1.7.1. Place the shake flask into the flask holders in the shaking incubator.

8.1.7.2. Set shaking incubator to 37°C and 200 RPM, turn on just the temp switch.

8.1.7.3. Incubate for 24-48 hours.

8.1.7.4. Check for contamination. If contaminated, add bleach and dispose down the drain.

8.2. **Inoculation**

8.2.1. Set up Equipment and Supplies:

8.2.1.1. Set water bath (Belly Dancer) to 37°C.

8.2.1.2. Set shaking incubator to 37°C and 200 RPM, turn on just the temp switch.

8.2.1.3. Pre-warm shake flask containing 100mL LB/AMP/ARA medium at 37° C.

8.2.2. Thaw the 1mL Vial of Frozen *E.coli* cells.

8.2.2.1. Remove the E.coli vial from storage in the -86° C freezer.

8.2.2.2. Thaw contents rapidly by agitation in a 37°C water bath (Belly Dancer).

8.2.3. Inoculate thawed *E.coli* Cells into 100mL of *E.coli* Cell Culture Medium.

8.2.3.1. Wipe off the 1mL vial with 70% isopropanol.

8.2.3.2. Using a sterile 2mL pipet, aseptically transfer the entire contents of the 1mL vial of thawed *E.coli* cells into the previously prepared shake flask containing 100mL LB/AMP/ARA medium.

Title: Batch Culture of *Escherichia coli* Recombinant for Jellyfish Green Fluorescent Protein SOP

8.2.3.3. Swirl to mix.
8.2.3.4. Immediately take a sample.

8.3. **Sampling**

8.3.1. **Set up equipment and supplies.**

8.3.1.1. Turn on spectrophotometer to read *E.coli* (550nm) per the spectrophotometer SOP.
8.3.1.2. Autozero the spectrophotometer with LB broth in the bottle per the spectrophotometer SOP.
8.3.1.3. Gather the following items on a clean lab bench:
micropipettors (100µL and 1000µL volumes) and sterile pipet tips
2mL pipets (>15) and pipet pump
bunsen burner
cell spreader
isopropanol in a beaker (~ 200mL)
15 spectrophotometer cuvettes
beaker containing sterile 1.5mL microfuge tubes
LB/ARA/AMP plates placed on clean lab bench away from working area

8.3.2. Aseptically withdraw 2 - 2.2mL of *E.coli* culture and place into cuvette. Cover cuvette with a small piece of laboratory film. Begin countdown to next sample.
8.3.3. Return suspension culture to the shaking incubator, making sure to turn on shaker and check temperature.
8.3.4. Take OD reading of sample at 550nm using the spectrophotometer.
Reminder: Invert the covered cuvette, before taking reading.
Reminder: If the initial sample OD reading is greater than 1.0, the sample should be diluted until it reads below 1.0 and then multiply by the dilution factor to obtain the absorbance value.
8.3.5. **Prepare serial dilutions for plating**
Note: Mix cells before each transfer.

8.3.5.1. For each time point label the bottom of 6 plates (as small as possible on the EDGE of the plates): [date], [product lot #], [time point], [dilution factor representing: **10^3**, **10^4**, **10^5**, **10^6** dilutions], [operator name].
8.3.5.2. Place 6 sterile microfuge tubes in a rack.
8.3.5.3. Label the tubes with the appropriate time point (t_{0min} or t_{60min} or t_{120min} etc).
8.3.5.4. Label each tube with a dilution factor (10, 10^2, 10^3, 10^4, 10^5, 10^6).
8.3.5.5. Add 900µL LB Broth from the bottle into EACH microfuge tube.
8.3.5.6. Add 100µL of sample from cuvette to the 10 dilution tube. Cap and mix well by inverting several times.
8.3.5.7. Change pipet tip and add 100µL of 10 dilution tube to the 10^2 tube. Cap and mix well by inverting several times.
8.3.5.8. Change pipet tip and add 100µL of 10^2 dilution tube to the 10^3 tube. Cap and mix well by inverting several times.
8.3.5.9. Change pipet tip and add 100µL of 10^3 dilution tube to the 10^4 tube. Cap and mix well by inverting several times.

Title: Batch Culture of *Escherichia coli* Recombinant for Jellyfish Green Fluorescent Protein SOP

8.3.5.10. Change pipet tip and add 100 µL of 10^4 dilution tube to the 10^5 tube. Cap and mix well by inverting several times.
8.3.5.11. Change pipet tip and add 100 µL of 10^5 dilution tube to the 10^6 tube. Cap and mix well by inverting several times.
8.3.6. Spread 200µl of each dilution (10^3 – 10^6) onto an LB/AMP/ARA plate (a total of four plates for each time point sample). Allow fluid to absorb into the media before turning plate over.
8.3.7. Incubate plates overnight in 37°C incubator.
8.3.8. Obtain pH reading for *E.coli* sample using pH meter per the pH meter SOP.
8.3.9. Repeat all of the sampling steps approximately every 60 minutes.
8.3.10. Determine viable cell concentration using the spread plates after overnight incubation.
8.3.10.1. Count colonies on appropriate plate for each time point.
8.3.10.2. Select a plate from each time point that has between 30 and 300 colonies.
8.3.10.3. Divide the number of colonies counted by the volume of sample spread on the plate to get the concentration of cells/µL and multiply by 1000 to get the cells/mL.
8.3.10.4. Multiply the number of cells/mL by the appropriate dilution factor to get the cell number per milliliter (cells/mL) in the original sample.
8.3.11. Identify cells as Gram Positive or Gram Negative
8.3.11.1. Perform Gram stain on one colony from each time point per Gram stain SOP

8.4. **Evaluate production of GFP.**
8.4.1. Place one plate from each time point on the UV light and observe if the colonies fluoresce.

8.5. **Prepare Growth Curve**
8.5.1. Plot OD, Viable Cell Number, and pH vs. Time on one graph.
8.5.2. Attach Growth Curve to the Batch Record.

8.6. **Calculate Growth Rate (u) and Doubling Time (Td)**
8.6.1. $u = (\ln OD_2 - \ln OD_1)/T_2 - T_1$
8.6.2. $Td = \ln 2/u$
8.6.3. Attach calculations to the Batch Record

9. Attachments:
9.1. Data table

10. History:

Name	Date	Amendment
S. Wallman	1997	Initial Release
E. Raitt, Deb Audino	2005	Put into 2005 SOP format, removed addition of NaOH to broth and agar, increased number of plates made, increased shaking speed, removed use of ice bucket, increased volume of sample for spread plate
Deb Audino, Kari Britt	05Sep06	Moved preparation of ampicillin after the autoclave step. LB culture section: add ARA to shake flask before removing 100mL to bottle.

Title: Batch Culture of *Escherichia coli* Recombinant for Jellyfish Green Fluorescent Protein SOP

Deb Audino	18 Apr07	Put effective date into new format. Separated inoculation and sampling sections.

TIME POINT (min)	OD (550nm)	pH	Colony Count on 10^3 plate	Colony Count on 10^4 plate	Colony Count on 10^5 plate	Colony Count on 10^6 plate	VIABLE CELL COUNT (cells/mL)	Gram (-) Gram (+)	GLOWING (YES/NO)
T_0									
T_{60}									
T_{120}									
T_{180}									
T_{240}									
T_{300}									
T_{360}									
T_{420}									
T_{480}									
T_{540}									
T_{600}									
T_{660}									

Title: Filter Integrity Testing Protocol

Approvals:
Preparer: __Kari Britt_____Date ____03Aug10_____
Reviewer: __Sonia Wallman_____Date____03Apr10_____

1. **Purpose:**
   1.1. Test the membrane integrity of used syringe filters.
2. **Scope:**
   2.1. Applies to previously used syringe filters to confirm sterility of filtered solutions.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:** N/A
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. small beaker
   7.2. 250 mL deionized water
   7.3. 7-10 inch silicone tubing
   7.4. pressure gauge
   7.5. 30mL syringe
   7.6. 0.22µm or 0.45µm syringe filter being tested
8. **Procedure:**
   8.1. Gather the following; small beaker, 250mL of deionized water, 7-10 inch silicone tubing, pressure gauge, a 30mL syringe, and the used syringe filter being tested.
   8.2. Take the 30mL syringe and fill it with deionized water.
   8.3. Attach the 30mL syringe to the filter.
   8.4. Push water through until the filter is completely wet.
   Note: If filter is not completely wet there will be no resistance for the pressure gauge to read.
   8.5. Detach the syringe from the filter.
   8.6. Refer to Figure 1 for bubble point assembly and perform the following steps.
      8.6.1. Draw air into the 30mL syringe, and then attach the syringe to the syringe adaptor on the pressure gauge so that it cannot come apart.
      8.6.2. Take the used syringe filter and attach it to the filter adaptor on the pressure gauge. Make sure it is twisted in tightly enough to prevent air from escaping, but do not over tighten.
      Note: Over tightening can result in the plastic threads being damaged.
      8.6.3. Attach a 7-10 inch piece of silicone tubing securely to the nozzle on the used syringe filter.
   8.7. Test the integrity of the syringe filter.
      8.7.1. Fill the small beaker with approximately 250mL of deionized water.

Title: Filter Integrity Testing Protocol

8.7.2. Place the end of the silicone tubing into the beaker so that it is submerged in the water.
8.7.3. Push the air from the syringe out, until the pressure gauge reaches 60psi for 0.22µm filter or 30psi for 0.45um filter.
8.7.4. When the designated pressure is reached on the pressure gauge a spurt of bubbles should emerge from tubing and into water.
8.7.5. If the pressure does not reach the designated psi level, then the filter is damaged.

9. Attachments:

9.1. Figure 1: Bubble Point Assembly
9.2. Figure 2: Pressure Gauge

10. History:

Name	Date	Amendment
Katrice Jalbert	25Mar06	Initial Release
Deb Audino	04Apr08	College name change
Kari Britt	03Aug10	Changed name of document from Bubble Point SOP to Filter Integrity Protocol. Also made formatting and grammar edits as needed throughout the document.

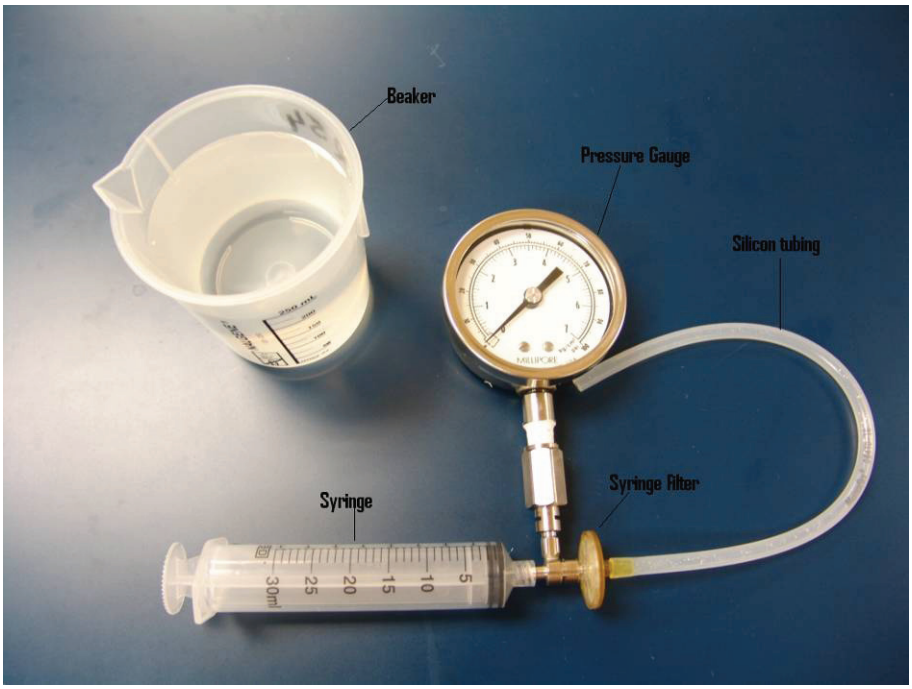


Figure 1: Bubble Point Assembly

Title: Filter Integrity Testing Protocol

Figure 2: Pressure Gauge

Great Bay Community College
320 Corporate Drive
Portsmouth, NH 03801

Title: Shimadzu UV-Visible Spectrophotometer SOP

Approvals:
Preparer: _____ Deb Audino _____ Date _____22Jul08_____
Reviewer:_____Bob O'Brien_____ Date _____22Jul08_____

1. **Purpose**:
   1.1. Operation of the Shimadzu UV-Visible Spectrophotometer.
2. **Scope:**
   2.1. This machine is used to measure how much light of a given wavelength is absorbed by a liquid sample.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/ lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Shimadzu UV-Visible Spectrophotometer manufacturer's instructions
5. **Definitions:**
   5.1. Cuvette: A small, transparent vessel. This is what a sample is put into for testing in the UV-Visible Spectrophotometer.
6. **Precautions:**
   6.1. N/A
7. **Materials:**
   7.1. sample to be tested
   7.2. cuvettes (visible or ultraviolet)
   7.3. lab tissue
   7.4. blank solution.
   7.5. 2mL pipettes
8. **Procedure:**
   8.1. Open panel door and make sure cuvette holders are empty, then close the panel door.
   8.2. Turn spectrophotometer "ON" by flipping the yellow switch on the side of the machine.
   8.3. The machine will automatically initialize and make a base line correction.
   8.4. Select **"8" CONDITION SET,** then press **ENTER**.
   8.5. Select **"5"** for **LAMP SELECT** to turn off UV light bulb, then press **ENTER.**
   8.6. Press the **FILE**, Key.
   8.7. Select one of the following numbers appropriate for the sample being tested.
      Select **"3"** for **E. COLI**
      Select **"7"** for **PICHIA**
      Select **"8"** for **CHO**
      Select **"10"** for **PROTEIN**
   8.8. Once you have made your selection press **ENTER.**
   8.9. It will then ask you for a **"Parameter Change Y/N**", Select **NO**, and press **ENTER**.

Title: Shimadzu UV-Visible Spectrophotometer SOP

8.10. Fill 2 of the same cuvettes each with about 2mL of blank solution. Hold the cuvette from the top to prevent tampering with the measurements, and wipe the sides with a lab tissue.
8.11. Open panel door and place the cuvettes with blank solution in the cuvette holders. **Make sure to use the appropriate orientation for the cuvettes you're using. Also make sure that the cuvettes used for the autozeroing are the same cuvette you use for the sample reading.** If using a standard cuvette, see fig 9.3, any orientation of the cuvette in the holder is acceptable, just make sure you wipe the cuvette's sides. If using a micro cuvetter, see figure 9.3, the microcuvette **MUST** be oriented in the holder so the the 1cm path length goes from left to right.
8.12. Press the **AUTO ZERO** key, then press **ENTER.**
8.13. When the the Auto Zero is complete, open the panel door and remove the **front** cuvette.
8.14. Do not replace cuvette in rear holder.
8.15. Using the same cuvette style, fill an empty cuvette with about 2-ml of the sample.
8.16. Clean the cuvette with a lab tissue.
8.17. Place in front cuvette holder, using the appropriate orientation and close the panel door.
8.18. Press **START** to take a reading.
8.19. Record the results or press **COPY** for a hard copy printout.
Note: If the initial sample OD reading is greater than 1.0, the sample should be diluted until it reads below 1.0 and then multiply by the dilution factor to obtain the absorbance value.
8.20. Open panel door and remove test sample from front cuvette holder.
8.21. To test additional samples: Place cuvettes in front holder and press start for a reading.
8.22. Record results, or press **COPY** for a hard copy printout.
8.23. Press **RETURN** to bring you back to step 8.9. Note: This will erase your old data.
8.24. Press **FILE** to return to the original screen.
8.25. Remove cuvettes remaining in holders.
8.26. Flip power switch located on the side, to turn off the machine.

9. Attachments:

9.1. Figure 1: Spectrophotometer
9.2. Figure 2: Spectrophotometer Control Panel
9.3. Figure 3: Micro and Standard Cuvettes

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial release
Margaret Stiles,	2005	into 2005 SOP format
Stephen A. Derdau,	28Sep05	Amended section 8.13 regarding orientation of cuvette, and added attachments. Modified step 8.11 added the original step 8.11 to 8.11, "wipe with Kim wipe."
Deb Audino	04Apr08	Changed Kimwipe to lab tissue. College name change

Title: Shimadzu UV-Visible Spectrophotometer SOP

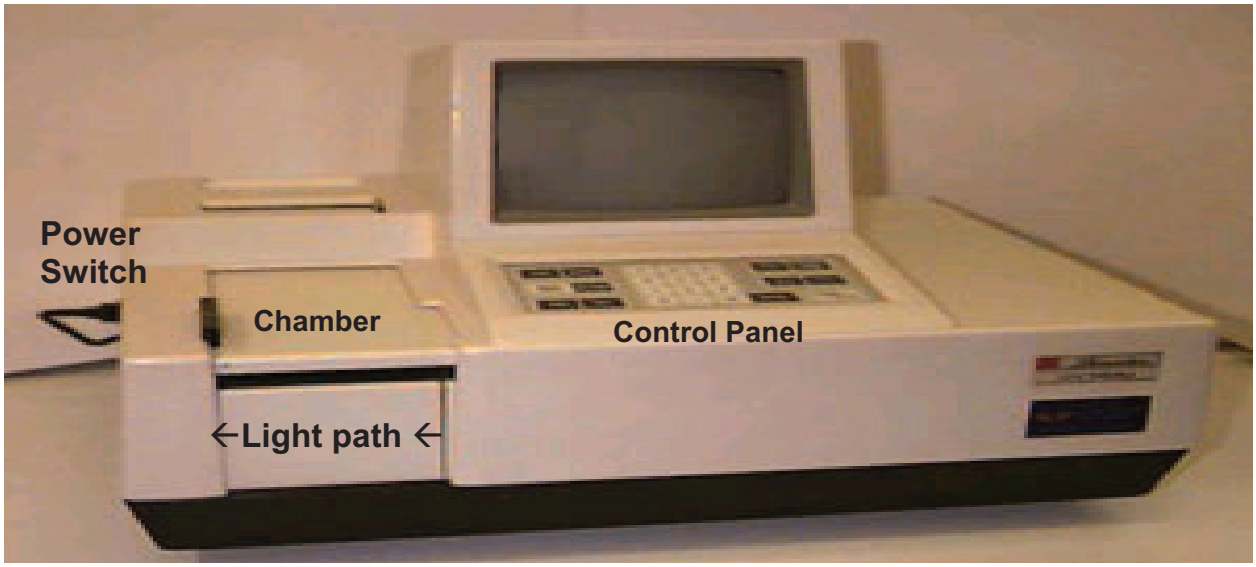


Figure 1: Spectrophotometer
Note: Light source runs from the right to the left through the sample in the cuvette chamber.

Figure 2: Spectrophotometer Control Panel

Title: Shimadzu UV-Visible Spectrophotometer SOP

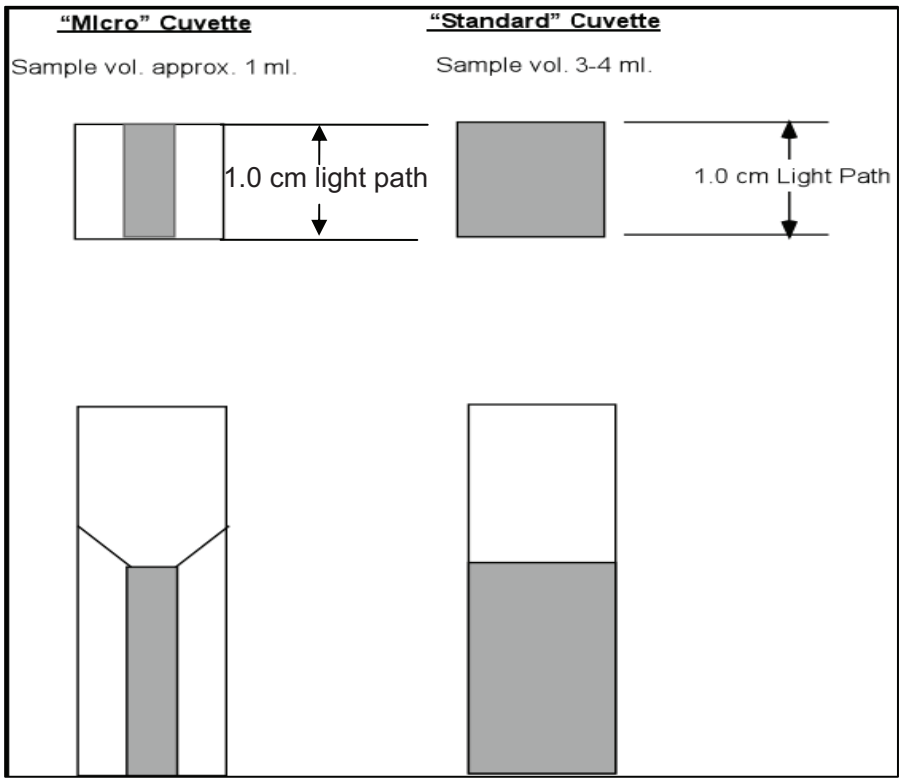


Figure 3: Micro and Standard Cuvettes
Gray Area = Sample
Image: http://bio.classes.ucsc.edu/bio100l/A%20SPECTROSCOPY/cuvettes.pdf

Title: Leica DME Microscope

Approvals:
Preparer: _____Kari Britt_____ Date __03Aug10_____
Reviewer:_____Sonia Wallman_____ Date __03Aug10_____

1. **Purpose:** Operation of the Leica DME microscope.
2. **Scope:** Applies to the proper usage of the Leica DME microscope.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Leica DME instruction manual
   4.2. autoclave SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Use care when handling biological materials. Wear gloves at all times.
7. **Materials:**
   7.1. Leica microscope
   7.2. plastic protective cover
   7.3. power cord
   7.4. immersion oil
   7.5. specimen slide
   7.6. cover slips
   7.7. 70% IPA
   7.8. lab tissue
   7.9. lab towels
   7.10. sharps container
   7.11. biohazard container
   7.12. autoclave
8. **Procedure:**
   8.1. **Operation**
      8.1.1. Always use your microscope on a hard stable secure surface.
      8.1.2. Remove plastic protective dust cover from microscope.
      8.1.3. Verify that the power cord is plugged into an appropriate power source if necessary. Refer to Figure 1.
      8.1.4. Turn on illumination for the microscope by rotating the illumination power switch on the bottom left side of microscope by turning it towards the operator. Refer to Figure 1.
      8.1.5. Set the illumination control to the lowest setting.
      8.1.6. Fully open the aperture diaphragm of the condenser by rotating the ring to the right 40X. Refer to Figure 3.

Title: Leica DME Microscope

8.1.7. Using the substage condenser focusing knob, raise the condenser to the top of its excursion. Refer to Figure 3.
8.1.8. Verify that the 4X objective is in the working position by rotating the revolving nose piece, until the 4X objective is in the working position. Refer to Figure 2.
8.1.9. Utilize the X axis stage knob by turning it clockwise to bring the stage forward to allow the specimen to be placed on the stage. Refer to Figure 2.
8.1.10. Open the specimen slide clips. Refer to Figure 3.
8.1.11. Place specimen slide onto the stage in the specimen clips.
8.1.12. Verify that the specimen is properly placed in the specimen clips and that the clips are closed, holding the specimen in place.
8.1.13. Utilize the X axis stage knob to align the specimen under the objective and over the light path of the condenser. Refer to Figure 2.
8.1.14. Utilize the Y axis adjustment knob for the specimen clamp to bring the specimen under the objective and over the light path of the condenser. Refer to Figure 2.

8.2. **Focusing**

8.2.1. Adjust the interpupillary distance of the eyepiece.
8.2.2. Adjust the eye piece to allow the operator to look through the eyepieces. Refer to Figure 1.
8.2.3. Looking through the right eye piece with your right eye only, close the left eye, turn the course adjustment knob to its position to raise the stage to bring the specimen into focus. Refer to Figure 2.
8.2.4. Looking through the left eye piece with your left eye only and adjust the diopter adjustment ring to focus the specimen. Refer to Figure 1.
8.2.5. Using the fine adjustment knob to raise the stage to bring the specimen into focus. This should only require ± 1.5 rotations of focus adjustment. Refer to Figure 2.
8.2.6. The specimen slide can be scanned by utilizing the X axis adjustment knob in combination with the Y axis adjustment knob for specimen clamp to bring different parts of the specimen under the objective and into view.
8.2.7. Rotate the nose piece so that the 10X objective is in the working position.
8.2.8. Refocus by using the fine adjustment knob to raise the stage to bring the specimen into focus. This should only require ±1.5 rotations of focus adjustment.

8.3. **Immersion Oil**

8.3.1. Focus the object on the specimen slide with a lower power objective.
8.3.2. Rotate the revolving nose piece to the so that the 40X objective is out of the way. Note: Do not allow oil to touch any objective beside the 100X.
8.3.3. Place a single drop of immersion oil on the slide directly above where the light is shining through the specimen slide. (Since air bubbles in the oil will impair the image of the object, make sure the oil is free of bubbles.)
8.3.4. Rotate the revolving nose piece to allow the 100X objective to come into place Note: The lens should go into the drop of immersion oil and *not hit the specimen slide*.
8.3.5. Looking through the oculars, you may need to increase the amount of light by turning the condenser to 100X or it may be necessary to turning the illuminator knob.

Title: Leica DME Microscope

8.3.6. Your object on the specimen slide should still be in the field of vision, but slightly out of focus. Use the fine adjustment knob as necessary to clearly focus the object on the specimen slide.
8.3.7. Once you're in the immersion oil **do not go back to the 40X objective**. If you go back, the 40X objective will get oil on it, which will damage the lense.
8.3.8. When you have completed your observation of the specimen, rotate the revolving nose piece to allow the 4X objective to come into the working position.
8.3.9. Utilize the X axis stage knob by turning the knob in a clockwise direction to allow the removal of the specimen.
8.3.10. Remove the specimen slide from the microscope and dispose of the specimen slide in the appropriate waste container. (Microscope slides with microbial organisms shall be placed in biohazard waste container).
8.3.11. The 100X lens should be cleaned of the residual oil. This can be accomplished by spraying a lab tissue with 70% IPA and gently wiping the lens with the tissue. Then take a dry lab tissue, and, with very light pressure, wipe the 100X objective lens.
8.3.12. Dispose of lab tissue in the appropriate waste container. (Lab tissues that may have microbial organisms shall be placed in biohazard waste container).

8.4. **Cleaning and Storage**
8.4.1. After each usage clean the microscope.
8.4.2. Lightly spray a lab towel with 70% IPA and wipe the external surface of the microscope with the lab towel. Finish at the stage area.
8.4.3. Dispose of the lab towel in the biohazard waste container.
8.4.4. Allow any residual IPA to evaporate.
8.4.5. Rotate the revolving nose piece to allow the 4X objective to be placed in the working position.
8.4.6. Place the plastic protective cover over microscope.

8.5. **Transporting microscope**
8.5.1. Loosely wrap the electrical cord around the arm of the covered microscope. Keep the cord from coming into contact with the stage.
8.5.2. Pick up the microscope with one hand on the arm and the other hand placed under the base of the scope.
8.5.3. Place the microscope securely on a cart for transport.

9. Attachments
9.1. Figure 1: Leica DME microscope left side component view
9.2. Figure 2: Leica DME microscope right side component view
9.3. Figure 3: Leica DME microscope front component view
9.4. Equipment log sheet 4.4.3

10. History:

Name	Date	Amendment
Bob O'Brien	18May07	Initial Release
Bob O'Brien	04Apr08	College name change
Kari Britt	03Aug10	Made formatting and grammar edits as needed.

Title: Leica DME Microscope

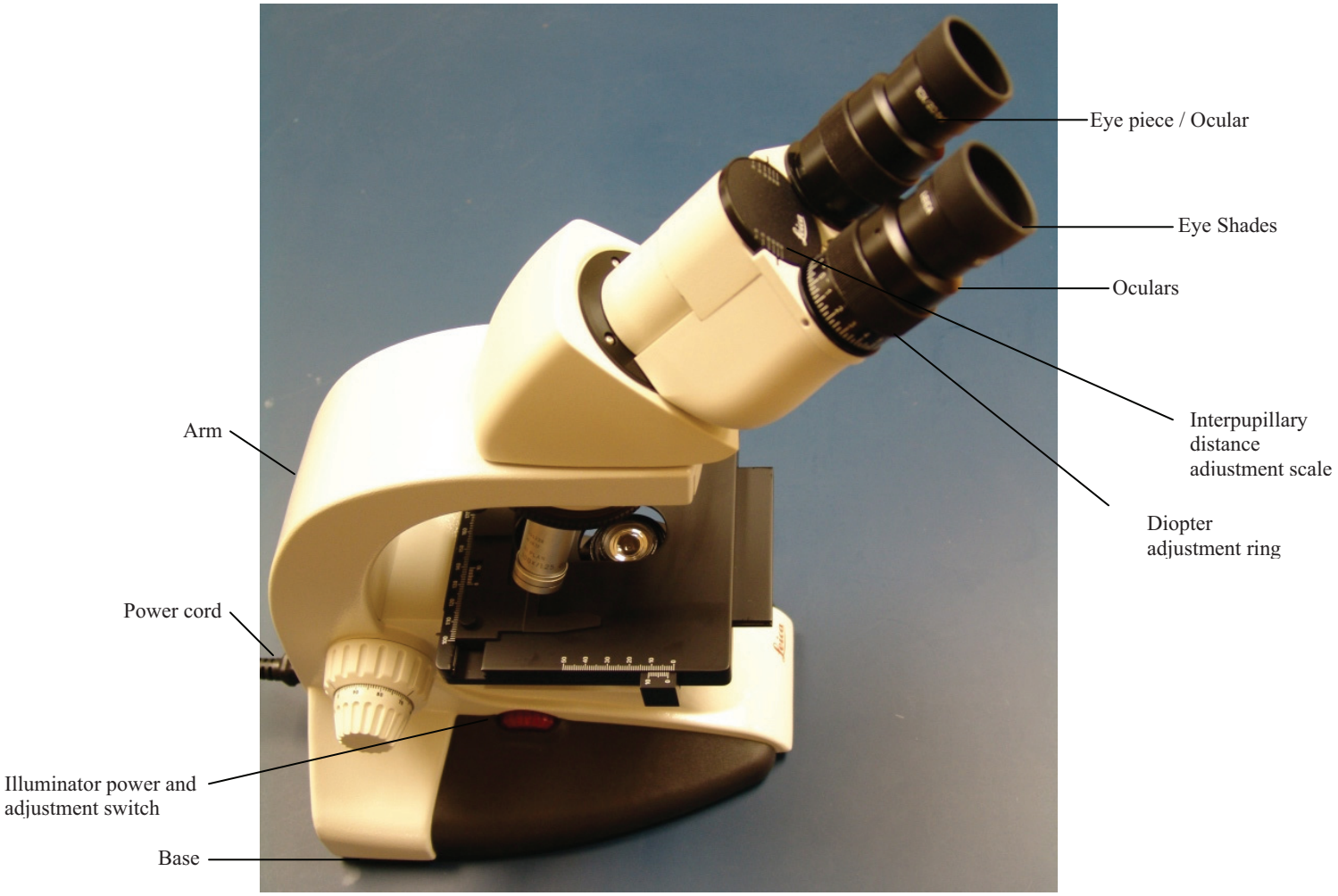


Figure 1: Leica DME microscope left side component view

Title: Leica DME Microscope

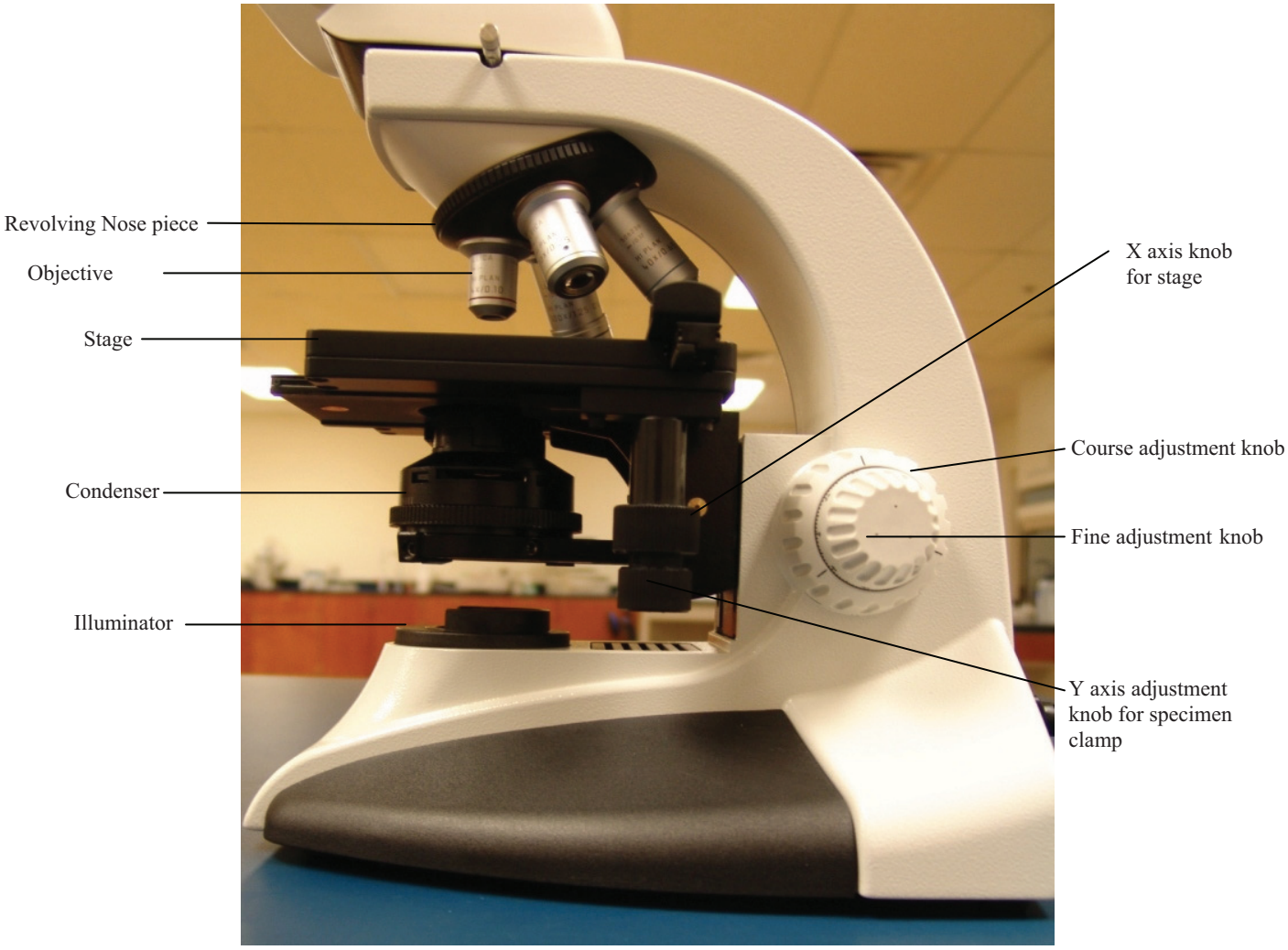


Figure 2: Leica DME microscope right side component view

Title: Leica DME Microscope

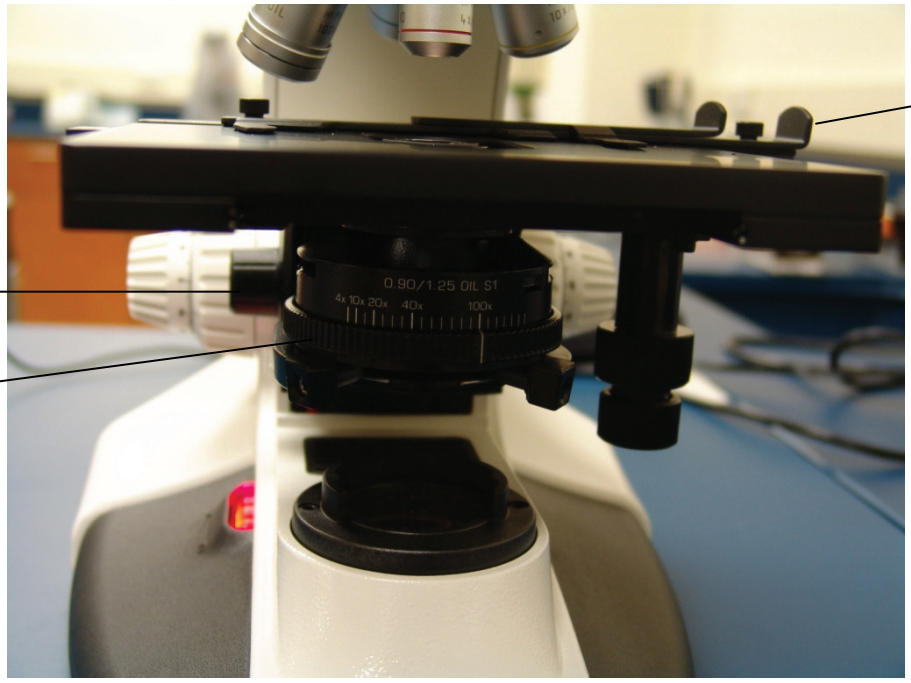


Figure 3: Leica DME microscope front component view

Batch Record: GFP Production from *Escherichia coli* Upstream Process

GFP Lot Number _____

Record Keeping Standards:

For each step in the batch record: the operator of the task will enter their initials (each operator has their own unique set of initials) and the date in the appropriate section(s) of the batch record. Another operator must initial and date in the appropriate section of the batch record to verify that the task was completed per SOP. No operator will verify their own work at any point. "If you didn't document it, you didn't do it!"

Batch records will be completed in blue or black ball point pen ONLY, and must be legible.

Any errors on a batch record will be crossed out with a single line through the error with the initials of the operator and the date. Corrections will be written in next to the crossed out error.

Use the following format to record dates: DDMMMYY. For July 10, 2006 use 10JUL06.

Use the 24 hour clock or "military time" to record time: 3:00pm would be written as 15:00.

Any and all deviations from a protocol or SOP, including abnormal results or retests performed, will be entered into the comments section at the end of each batch record. Be as detailed and specific as possible, include all steps taken before and/or after an abnormal reading, and provide an explanation for any deviations from a step.

Batch Record: GFP Production from *Escherichia coli* Upstream Process

GFP Lot Number _____

1. Prepare Media Broth Culture Broth: Luria-Bertani (LB) Broth, Ampicillin (0.09mg/mL), Arabinose (1.8mg/mL)		
Weigh out approximately 4g of LB Broth premix. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Weigh out approximately 0.36g of arabinose powder. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Add LB broth premix and arabinose to a clean 500mL shake flask. Flask I.D. number:_____	Operator/Date	Verifier/Date
Measure about 200mL of deionized water using a 250mL graduated cylinder and add it to the 500mL shake flask. Stir to dissolve. Volume of DI water measured:_____mL	Operator/Date	Verifier/Date
Measure approximately 100mL of the LB/ARA broth using the 250mL graduated cylinder and transfer it to the 125mL glass bottle. Volume of LB Broth transferred from shake flask to bottle:_____mL	Operator/Date	Verifier/Date
Autoclave per autoclave SOP for 20 minutes at 121°C. Autoclave I.D. #:_____ Time:_____minutes Temperature:_____ºC Pressure:_____ psi	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: GFP Production from *Escherichia coli* Upstream Process

GFP Lot Number _____

2. Prepare Culture Agar Luria-Bertani (LB) Agar, Ampicillin (0.09mg/mL), Arabinose (1.8mg/mL)		
Weigh out approximately 35g of LB Agar (premix): Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Weigh out approximately 1.8g of arabinose powder: Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Measure approximately 1L of deionized water using a 1L graduated cylinder and add to the 2L Erlenmeyer flask. Stir to mix. Volume of DI water measured:_____L	Operator/Date	Verifier/Date
Autoclave per autoclave SOP for 20 minutes at 121°C. Autoclave I.D. #:_____ Time:_____minutes Temperature:_____ºC Pressure:_____ psi	Operator/Date	Verifier/Date
Remove from autoclave when pressure reaches <5psi and <80°C. **Cool** in a 55ºC water bath.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: GFP Production from *Escherichia coli* Upstream Process

GFP Lot Number _____

3. Prepare Ampicillin Solution and Add to Media Broth and Agar Ampicillin Stock solution: 6mg/mL		
Weigh out approximately 0.120g of ampicillin powder and place in small beaker. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Measure approximately 20mL deionized water with a 25mL graduated cylinder and add to the beaker containing the ampicillin. Swirl to dissolve. Volume of DI water measured:_____mL	Operator/Date	Verifier/Date
Sterilize filter solution using sterile 0.2 µm syringe filter as per the SOP: <u>Filter information:</u> Manufacturer:_____ Catalog number: _____ Lot number: _____	Operator/Date	Verifier/Date
Evaluate the integrity of the filter by performing the bubble point test per the Bubble Point Test SOP. Pressure needed to generate a steady flow of bubbles: _____psig Does the filter pass the test (pressure >45psig): YES/NO (circle one)	Operator/Date	Verifier/Date
Aseptically add approximately 1.5mL of sterile filtered ampicillin stock solution (6mg/mL) to shake flask containing 100mL culture medium (Media must be cool to the touch!), swirl to mix. Volume of Ampicillin added: _____mL	Operator/Date	Verifier/Date
Aseptically add 15mL of sterile filtered ampicillin stock solution (6mg/mL) to the 2L flask containing 1L agar medium, swirl to mix. Volume of Ampicillin added: _____mL	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: GFP Production from *Escherichia coli* Upstream Process

GFP Lot Number _____

4. Pour Culture Plates/Proof LB Broth		
Pour agar plates and allow agar to solidify undisturbed with covers on as per SOP.	Operator/Date	Verifier/Date
Check for contamination. Autoclave and discard any plates showing microbial growth. Total number of plates:_____ Number of plates with contamination:_____	Operator/Date	Verifier/Date
Place the shake flask into the flask holders in the shaking incubator and incubate at 37 ºC and 200rpm. Incubator ID:_____ Temperature: _____ ºC Shaking Speed:_____ RPM Incubation Time: _____ hours	Operator/Date	Verifier/Date
Check for contamination. If contaminated, autoclave and dispose down the drain. Contamination: yes / no (circle one)	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: GFP Production from *Escherichia coli* Upstream Process

GFP Lot Number _____

5. Inoculation		
Prewarm shake flask containing 100ml LB/AMP/ARA medium at 37 ºC.	Operator/Date	Verifier/Date
Remove the *E.coli* vial from storage in the -86 ºC freezer. Vial ID:_____	Operator/Date	Verifier/Date
Thaw contents rapidly by agitation in a 37 ºC water bath.	Operator/Date	Verifier/Date
Aseptically transfer the *E.coli* cells into the previously prepared shake flask per the SOP and immediately take a sample.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: GFP Production from *Escherichia coli* Upstream Process

GFP Lot Number _____

TIME POINT (min)	OD (550nm)	pH	Operator/Date	Verifier/Date
0				
60				
120				
180				
240				
300				
360				
420				
480				
540				
600				
660				
720				

Batch Record: GFP Production from *Escherichia coli* Upstream Process

GFP Lot Number _____

Time Point (min.)	Colony Count on 10^3 plate	Colony Count on 10^4 plate	Colony Count on 10^5 plate	Colony Count on 10^6 plate	Viable Cell Count (cells/ml)	Gram (-) Gram (+)	Glowing (Yes/No)	Operator/Date	Verifier/Date
0									
60									
120									
180									
240									
300									
360									
420									
480									
540									
600									
660									
720									

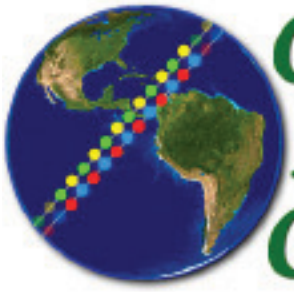
*** Attach growth curve and double time calculation.**

Manufacturing Technician (Upstream)

REF	Key Functions & Tasks (Upstream Manufacturing Technician)
1	**Work in compliance with EH&S.**
1.a	Wear appropriate personal protective equipment.
1.b	Work in controlled environments.
1.c	Participate in emergency drills and emergency response teams.
1.d	Identify unsafe conditions and take corrective action.
1.e	Appropriately and safely access production equipment.
1.f	Handle, label, and dispose of hazardous / biohazard materials.
1.g	Access and utilize MSDS.
1.h	Perform permitting procedures.
1.i	Carries out operations with attention to OSHA and EPA regulations, and other applicable state and federal regulations.
1.j	Keeps work areas clean and safety equipment in order.
1.k	Participate in all company safety training and audits as required.
1.l	Assists with waste treatment operations.
2	**Work in compliance with cGMPs.**
2.a	Assists in environmental monitoring activities.
2.b	Follow SOPs for all operations.
2.c	Records process data and completes batch records as required.
2.d	Maintain equipment logbooks.
2.e	Control and receipt of raw materials.
2.f	Maintain training documentation.
2.g	Maintain equipment and processes in a validated state.
2.h	Working in controlled/classified areas (gowning, aseptic technique).
2.i	Ensure appropriate flow of personnel, equipment, and materials.
2.j	Change control for process, equipment, and documentation.
2.k	Label and apply status to equipment and materials.
2.l	Identify and report exception events and CAPA.
3	**Clean and maintain production areas.**
3.a	Housekeeping / pest control.
3.b	Sanitize and clean of controlled spaces.
3.c	Preparation of cleaning materials and solutions.
3.d	Assist in environmental monitoring for routine and changeover operations.
3.e	Document cleaning.
4	**Maintain effective communication.**
4.a	Deliver shift change update.
4.b	Communicate with coworkers and/or customers to ensure production or service meets requirements.
4.c	Suggest continuous improvements.
4.d	Coordinate with work teams / internal customers.
4.e	Maintain security and confidentiality.
4.f	Respond appropriately to internal auditors and external inspectors.
4.g	Assist in writing, reviewing, and commenting on technical documents.

REF	Key Functions & Tasks (Upstream Manufacturing Technician)
5	**Prepare process materials.**
5.a	Weigh, dispense, and label raw materials for use in production.
5.b	Dispense consumables and intermediates.
5.c	Control and reconcile inventory with enterprise control system (MRP, SAP, manual database).
5.d	Prepare and sterilize buffers and solutions.
5.e	Sample and test buffers and solutions.
5.f	Transfer buffers and solutions to use point.
5.g	Prepare filters for use.
5.h	Prepare, pasteurize / sterilize, and titrate media and feed solutions.
5.i	Manage chromatography resins.
6	**Prepare equipment.**
6.a	Clean CIP vessels, transfer lines, and filter trains .
6.b	Clean COP equipment (or sonicator).
6.c	Depyrogenate components and equipment.
6.d	Sterilize SIP vessels, transfer lines, and sampling ports.
6.e	Perform pressure test.
6.f	Prepare and assemble components and equipment.
6.g	Autoclave components and equipment.
6.h	Perform WFI flush of transfer lines.
6.i	Perform scheduled sanitizations of hoods.
6.j	Prepare and standardize probes and ancillary instruments.
6.k	Prepare, assembly, and integrity test filters.
6.l	Visually inspect equipment.
6.m	Maintain equipment logs and status tags.
6.n	Complete, review and approve equipment process records.
7	**Perform basic manufacturing operations.**
7.a	Perform processes following batch records, validation protocols, and/or SOPs.
7.b	Maintains and controls processes in an automated control environment.
7.c	Record process data.
7.d	Inspect materials at all stages of process to determine quality or condition.
7.e	Participate in the installation, modification, and upgrade of equipment.
7.f	Operate, monitor, and maintain equipment, tools, and workstation.
7.g	Recognize and respond appropriately to atypical events.
7.h	Participate in troubleshooting and root cause analysis of operations.
8	**Perform upstream manufacturing operations.**
8.a	Work in an aseptic environment (laminar flow hood / biosafety cabinet / cleanrooms).
8.b	Perform vial thaw from a working cell bank.
8.c	Perform cell culture expansion.
8.d	Monitor cell concentration by cell counting or measuring OD.
8.e	Inoculate seed reactor.
8.f	Transfer of seed culture to production reactors.
8.g	Monitor and control growth of cells in batch, fed-batch, and perfusion reactors.
8.h	Perform aseptic additions of media, solutions, and/or gases to reactors.
8.i	Perform CIP/SIP of bioreactors.
9	**Perform Sampling.**
9.a	Prepare sample port for aseptic sampling.
9.b	Obtain in-process samples according to batch records or sampling plans.
9.c	Label samples appropriately.
9.d	Record sample collection and distribution (storage and chain of custody).
9.e	Perform in-process chemical and/or microbiological tests.

Upstream - tPA



GLOBAL
BIOMANUFACTURING
CURRICULUM

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Title: Batch Culture of Recombinant tPA Secreting CHO Cells SOP

Approvals:
Preparer:___Deb Audino_____Date _____04Apr08_____
Reviewer:__Bob O'Brien_____ Date _____04Apr08_____

1. **Purpose:**
   1.1. To produce a batch culture of mammalian cells.
2. **Scope:**
   2.1. Applies to the production of human tissue plasminogen activator (tPA) protein from recombinant Chinese Hamster Ovary (CHO) cells.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. ATCC CRL9606 growth guidelines
   4.2. 100mL Spinner Flask SOP
   4.3. Biological Safety Cabinet SOP
   4.4. CO_2 Incubator SOP
   4.5. pH Meter SOP
   4.6. spectrophotometer SOP
   4.7. Biolyzer SOP
   4.8. Biolyzer Pipet SOP
   4.9. Trypan Blue Assay SOP
   4.10. tPA ELISA SOP
   4.11. tPA Activity Assay SOP
   4.12. Applikon Bioreactor SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Use BL2 safety measures and discard waste in biohazard containers.
7. **Materials:**
   7.1. biological safety cabinet
   7.2. vial of CHO cells (ATCC 9606-CRL) recombinant for human tissue plasminogen activator (tPA)
   7.3. Ham's F12 Medium
   7.4. fetal bovine serum (FBS)
   7.5. 10X PBS
   7.6. 100mL vessel
   7.7. 1M $NaHCO_3$ (sodium bicarbonate)
   7.8. ProCHO4 media (manufactured by Cambrex/Biowhittaker)
   7.9. 200mM glutamine
   7.10. 10mg/mL gentamycin
   7.11. sterile 100mL Bellco spinner flasks

Title: Batch Culture of Recombinant tPA Secreting CHO Cells SOP

7.12. sterile transfer pipets (50mL, 10mL, and 2mL) and pipettor
7.13. CO_2 incubator containing magnetic stir plate
7.14. UV-visible recording spectrophotometer
7.15. cuvettes for spectrophotometer
7.16. 1.5mL microfuge tubes
7.17. microscope with 1000x magnification
7.18. cryogenic vials (1mL capacity) for storage of CHO cell master/working cell bank
7.19. sterile 250mL glass bottles for storage of CHO cell media
7.20. computer and Microsoft Excel for Windows
7.21. 100 mL glass bottle
7.22. Sigma 2K15 refrigerated centrifuge
7.23. biolyzer
7.24. biolyzer pipet

8. Procedure:

8.1. **Media Preparation:** Ham's F12 Medium, 90%; Fetal Bovine Serum, 10%:

8.1.1. Clean, assemble, and autoclave 100mL Bellco spinner flasks per SOP.

8.1.2. Gather the following items, spray with 70% isopropanol, and place in the biological safety cabinet:
automatic pipettor
10mL sterile pipettes
50mL sterile pipettes

8.1.3. Prepare biological safety cabinet (BSC) per SOP.

8.1.4. Spray the outside of the following with 70% isopropanol then place in the biological safety cabinet:
100mL sterile Bellco spinner flasks
500mL bottles of pre-sterilized Ham's F12 Medium
100mL of pre-sterilized, heat inactivated fetal bovine serum (FBS)

8.1.5. Sterilely remove 90mL ± 1mL of Ham's F12 Medium from a 500mL bottle of Ham's F12 and place in a sterile 100mL spinner flask.
8.1.5.1. Repeat with a second 100mL spinner flask.

8.1.6. Sterilely add 10mL ± 1 of FBS to the Ham's F12 bottle.
8.1.6.1. Repeat with the second 100mL spinner flask.

8.1.7. Label all spinner flasks as 90% Ham's F12, 10% FBS, [date], [group#], [operator initials].

8.1.8. Place all spinner flasks containing CHO cell media in the CO_2 incubator. Set the speed of the magnetic stirrer to the maximum setting that ensures an even mixing of the culture without foaming.
8.1.8.1. Verify that the temperature is 37 ± 0.5°C and percentage of CO_2 is 5 ± 0.5%.

8.1.9. Check media for contamination after a minimum of 24 hours.

8.1.10. Store media in refrigerator.

8.2. **Inoculation**

8.2.1. Pre-warm the spinner flasks containing CHO cell culture medium at 37° C ± 0.5°C.

Title: Batch Culture of Recombinant tPA Secreting CHO Cells SOP

8.2.2. Spray a minimum of four 2mL sterile pipettes with 70% isopropanol and place in the BSC.
8.2.3. Prepare Biological Safety Cabinet per BSC SOP.
8.2.4. Remove 2 vials of CHO cells from storage in the -86°C freezer.
8.2.5. Thaw contents rapidly by agitation in a 37°C ± 0.5°C water bath (Belly Dancer).
8.2.6. Spray vials with 70% isopropanol, and place in the biological safety cabinet.
8.2.7. Sterilely transfer the entire contents of each 1mL vial of thawed CHO Cells into each of the previously prepared Bellco Spinner Flask containing 100mL CHO Cell Culture Medium using a 2mL sterile pipet.
8.2.8. Swirl to mix.
8.2.9. Immediately after adding CHO Cells to Bellco spinner flask (day 0) and at 1-day intervals the culture will be sampled to determine the OD, pH, viable cell count, analyte levels and tPA concentration. The culture will be scaled up just before the exponential phase of the growth curve begins to slow down, indicating the cell culture is moving into the stationary phase of the growth curve. The live cell concentration should be approaching 1 million cells/mL.

8.3. **Sampling the Culture**

8.3.1. Collect the following items, spray with 70% IPA and place in Biological Safety Cabinet:
2 microfuge tubes labeled “tPA, Tn” and “cells”
1 microfuge tube holder
3 spectrophotometers cuvettes
1 cuvette holder
4 2mL pipets
pipet pump
8.3.2. Prepare biological safety cabinet per BSC SOP.
8.3.3. Prepare pH Meter per pH Meter SOP.
8.3.4. Prepare biolyzer and biolyzer pipet per biolyzer and biolyzer pipet SOPs.
8.3.4.1. Remove lactate and glucose Biolyzer slides from the -20° C freezer.
8.3.5. Prepare spectrophotometer per spectrophotometer SOP using media to zero the machine.
8.3.6. Spray blank and culture spinner bottle with 70% IPA and place in biological safety cabinet.
8.3.7. Using aseptic technique, remove 2 – 2.2mL sample of culture and place into a cuvette. Note: Do not mix blank and sample cuvettes.
8.3.8. Remove all items from the biological safety cabinet.
8.3.9. Return suspension culture and blank to the CO_2 incubator, making sure to loosen caps once in incubator. Set the speed of the magnetic stirrer to the maximum setting that ensures an even vortexing of the culture without foaming.
8.3.10. Cover the sample cuvette with parafilm.
8.3.11. Take OD Reading at 650nm per spectrophotometer SOP.
8.3.11.1. Mix CHO sample by inverting the cuvette several times before taking reading.
8.3.12. Take readings for glucose and lactate using the Biolyzer per the Biolyzer SOP.
8.3.13. Determine cell count using the Trypan Blue SOP.

Title: Batch Culture of Recombinant tPA Secreting CHO Cells SOP

8.3.14. Take pH reading per pH meter SOP.
8.3.14.1. Transfer the sample to a test tube to measure the pH per the pH meter SOP.
8.3.15. Remove the sample to a 1.5mL tube and centrifuge at high speed for 5minutes. Remove the supernatant and store at 2-8°C until needed.

8.4. **Scale up to 1L bioreactor**

Note: When the 100mL suspension culture of CHO cells reaches a concentration of about 1,000,000 cells/mL, the entire contents of the 100mL spinner flask will be added to the bioreactor containing 1L of CHO cell media.

8.4.1. Prepare 1M $NaHCO_3$ (sodium bicarbonate)
8.4.1.1. Weigh out 21 ± 1 grams of $NaHCO_3$ and transfer to an Applikon bioreactor feed bottle.
8.4.1.2. Label the bottle as 1M $NaHCO_3$, [date], [initials], [group number], storage: room temp, disposal: drain.
8.4.1.3. Using a 250mL graduated cylinder, measure 250 ± 5mL deionized water and transfer into the feed bottle.
8.4.1.4. Add a magnetic stir bar and stir on a magnetic stirrer to dissolve.
8.4.2. Prepare 1X PBS.
8.4.2.1. Using a 10mL pipet, measure 10 ± 0.5mL of 10x PBS and dispense into a 100mL vessel.
8.4.2.2. Using a 100mL graduated cylinder, measure 90 ± 5mL of deionized water and transfer into the 100mL vessel. Swirl to mix.
8.4.2.3. Label vessel as 1X PBS, [date], [initials], [group number], storage: room temp, disposal: drain.
8.4.3. Set up Applikon bioreactors per the bioreactor SOP including calibrating the pH probe.
8.4.4. Autoclave the bioreactors with 1X PBS per the bioreactor SOP for 20 minutes.
8.4.5. Remove the bioreactor vessel from the autoclave and connect the DO probe to the controller.
8.4.6. Turn on the controller.
8.4.7. Allow the DO probe to polarize for a minimum of 6 hours.
8.4.8. In the biological safety cabinet prepare the media.
8.4.8.1. Add ~6mL of 200mM glutamine (1.2mM final concentration) and 10mL of 10mg/mL gentamycin (0.1mg/mL final concentration) to the 1L bottle of ProCHO4 media.
8.4.9. Aseptically pour the media into the bioreactor through the inoculation port using a sterile funnel.
8.4.10. Connect the remaining parts of the bioreactor to the controller.
8.4.11. Input the setpoints and limits listed in the table below per the bioreactor SOP.

Parameter	pH	Temp (^{o}C)	%DO	Stirrer (rpm)
Setpoint	7.2	37	50	75
Upper Limit	7.3	38	52	76
Lower Limit	7.1	36	48	74

Title: Batch Culture of Recombinant tPA Secreting CHO Cells SOP

8.4.12. When the 100mL suspension culture of CHO cells reaches a concentration of approximately 1,000,000 cells/mL, aseptically transfer the culture to the bioreactor.
8.4.13. Start BioXpert Lite per bioreactor SOP.
8.4.14. Immediately and at 2-day intervals, sample the culture to determine OD, pH, viable cell count, analytes and tPA over time (see step 8.3).
8.5. When the cell count reaches approximately 1,000,000 cells/mL shut down the bioreactor per the bioreactor SOP and harvest cells as described below.
8.6. **Harvest and Preparation of Working Cell Bank**
8.6.1. Gather the following, spray with 70% IPA and place in the BSC:
30mL sterile centrifuge tubes (40)
25mL sterile pipets (10)
2mL sterile pipets (20)
cryovials (55)
250mL sterile glass bottles (4)
8.6.2. Prepare the biological safety cabinet per SOP.
8.6.3. Using a 25mL sterile pipet, transfer 25mL of the culture into sterile 30mL centrifuge tubes.
8.6.4. Centrifuge tubes for 10min. at 3000xg per centrifuge SOP.
Note: Always balance the test tubes in the centrifuge.
8.6.5. Prepare storage menstrum
8.6.5.1. Combine the following item into a container capable of holding >50mL and mix well.
40mL± 1.0mL of Ham's F12
5mL± 0.5mL of FBS
5mL± 0.5mL of glycerol
8.6.5.2. Filter sterilize.
8.6.5.3. Label bottle as CHO storage menstrum with the date.
8.6.5.4. Spray with 70% IPA and place in the biological safety cabinet.
8.6.5.5. Remove filter unit and place cap on bottle.
8.6.6. Following centrifugation of the culture, decant tPA containing medium into sterile 250mL bottles.
8.6.7. Label bottles as unpurified tPA in ProCHO4 [date} and [group #].
8.6.8. Store in the refrigerator at 2-8°C.
8.6.9. Add about 1mL of storage menstrum to each centrifuge tube to resuspend the pelleted CHO cells.
8.6.10. Sterilely dispense 1mL± 0.1mL aliquots into sterile 1.5mL cryovials.
50 cryovials for the working cell bank are expected.
8.6.11. Label in the following manner using a cryopen: CHO (ATCC CRL-9606) BT220-[day or evening], DATE.
8.6.12. Place in a styrofoam tube rack. Label container same as cryovials.
8.6.13. Store at -85°C.
8.7. **Determine tPA Concentration**
8.7.1. Determine the tPA concentration at each time point per tPA ELISA SOP.

Title: Batch Culture of Recombinant tPA Secreting CHO Cells SOP

8.7.2. Determine the activity of the tPA at each time point per tPA Activity Assay SOP.

8.8. **Prepare Growth Curves**

8.8.1. Plot OD, pH, viable cells, glucose, lactate, and tPA vs. time (use 2 y-axes).

8.8.2. Attach growth curve to Batch Record.

8.8.3. Determine growth rate and doubling time of the 100mL spinner flask and 1L bioreactor cultures.

8.8.4. Attach calculations to Batch Record.

9. Attachments:

9.1. Data table

10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
Deb Audino Ellery Raitt	6/2005	Put into 2005 SOP format. Removed trypan blue section and replaced with reference to Trypan Blue SOP. Added bioreactor section
Deb Audino	12Jan06	Removed 50mL and 500mL spinner flasks and replaced with 100mL spinner flasks. Reduce volume of storage menstrum added to cells from 2.5mL to 1mL
Deb Audino	05Mar08	Moved the polarization of the DO probe earlier in the procedure. Removed placing the bioreactor vessel in the BSC. Removed determne tPA activity.
Deb Audino	04Apr08	College name change

TIME (hours)	OD 650nm	pH	LIVE Cell Count	DEAD Cell Count	Viable Cells/mL	Percent Viability	GLU mg/dL	LAC mmol/L

Title: Biological Safety Cabinet (BSC) Operation SOP

Approvals:
Preparer:_____ Deb Audino _____ Date _____03Apr08_____
Reviewer: _____Bob O'Brien_____ Date _____03Apr08_____

1. **Purpose:**
   1.1. Operation of the Biological Safety Cabinet (BSC).
2. **Scope:**
   2.1. Applies to the use of the BSC for maintaining a sterile environment for media preparation, culture inoculation and culture sampling.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Biological Safety Cabinet Operator's Manual
   4.2. autoclave SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. UV Light is damaging to eyes and skin. Avoid exposure.
7. **Materials:**
   7.1. 70% Isopropanol (IPA) in spray bottle
   7.2. lab towels
   7.3. Tyvek sleeves
   7.4. gloves
8. **Procedure:**
   8.1. **UV Decontamination – Performed for initial use of the day**
      8.1.1. Put on gloves and Tyvek sleeves.
      8.1.2. Open sash **slightly** and **immediately** turn on blower.
      8.1.3. Spray and wipe down the stainless steel work surfaces of the BSC with 70% IPA.
      8.1.4. With gloved hands, spray all necessary materials not affected by UV with 70% IPA and place in BSC.
      8.1.5. Close the sash, turn off the blower, and turn on the UV light. This switch is located on the control panel. Refer to Figure 1.
      8.1.6. Leave the UV light on for at least 15 minutes.
      8.1.7. Place a biohazard waste receptacle adjacent to the cabinet.
      8.1.8. Once the appropriate time has elapsed, turn off the UV light.
      8.1.9. Turn on the visible light. This is located on the control panel.
      8.1.10. Turn on the receptacle power.
      8.1.11. Open the sash **slightly,** and **immediately** turn on the blower.
      8.1.12. After blower is on, raise the sash to the safe operating level.
      Note: The alarm will sound if the sash is raised above this level.

Title: Biological Safety Cabinet (BSC) Operation SOP

8.1.13. Label the BSC as "DECONTAMINATED".

8.2. **Operation**

8.2.1. Verify that the BSC has been decontaminated by noting the status label.
8.2.1.1.If the BSC is not labeled as "DECONTAMINATED", complete step 8.1.

8.2.2. Replace gloves and sleeves with new ones.

8.2.3. Spray down arms and hands with 70% IPA prior to entering the BSC.
Note: Allow 30 seconds for the 70% IPA to dry. This ensures disinfection.

8.2.4. Spray all necessary equipment that needs to go into the BSC with 70% IPA, and allow to dry for 30 seconds.

8.2.5. Place all necessary equipment inside of the BSC.

8.2.6. Perform protocol while working in center of the work surface.
Note: **Do not block the intake grills**. This ensures proper airflow. Refer to Figure 2.

8.2.7. Once the protocol is completed, remove all equipment from the BSC.

8.2.8. Place any disposable materials that have contacted any cellular organism into the biohazard waste receptacle.

8.2.9. Spray down the stainless steel work surfaces with 70% IPA. Allow 30 seconds for the IPA to dry, then wipe down stainless steel work surfaces with a lab towel. Spray down the work surfaces once more, but allow the IPA to dry without wiping.

8.2.10. Dispose lab towels used to clean BSC into the biohazard waste receptacle.

8.2.11. Turn off the visible light.

8.2.12. Leave the blower on and the sash open to the safe operating level.

8.3. **Cleaning - Performed after the final use of the day**

8.3.1. Wipe down the stainless steel work surfaces with 70% IPA.

8.3.2. Lower the sash, and turn off the blower.

8.3.3. Label the BSC as "NEEDS TO BE DECONTAMINATED".

8.3.4. Dispose lab towels used to clean the BSC into the biohazard waste receptacle, when container has reached appropriate level it will need to be autoclaved.

8.3.5. Prepare autoclave per autoclave SOP.

8.3.6. Autoclave the biohazard waste receptacle per autoclave SOP.

9. Attachments: N/A

9.1. Figure 1: Biological Safety Cabinet Control Panel

9.2. Figure 2: Biological Safety Cabinet Components

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial Release
Deb Audino	04July05	Put into 2005 SOP format, and added status labels.
Dan Borkush	29Sep05	Removed references to decontamination tags.
Bob O'Brien	21Jun07	Added photographs, re-worked process, and added autoclave reference.
Deb Audino	04Apr08	College name change

Title: Biological Safety Cabinet (BSC) Operation SOP

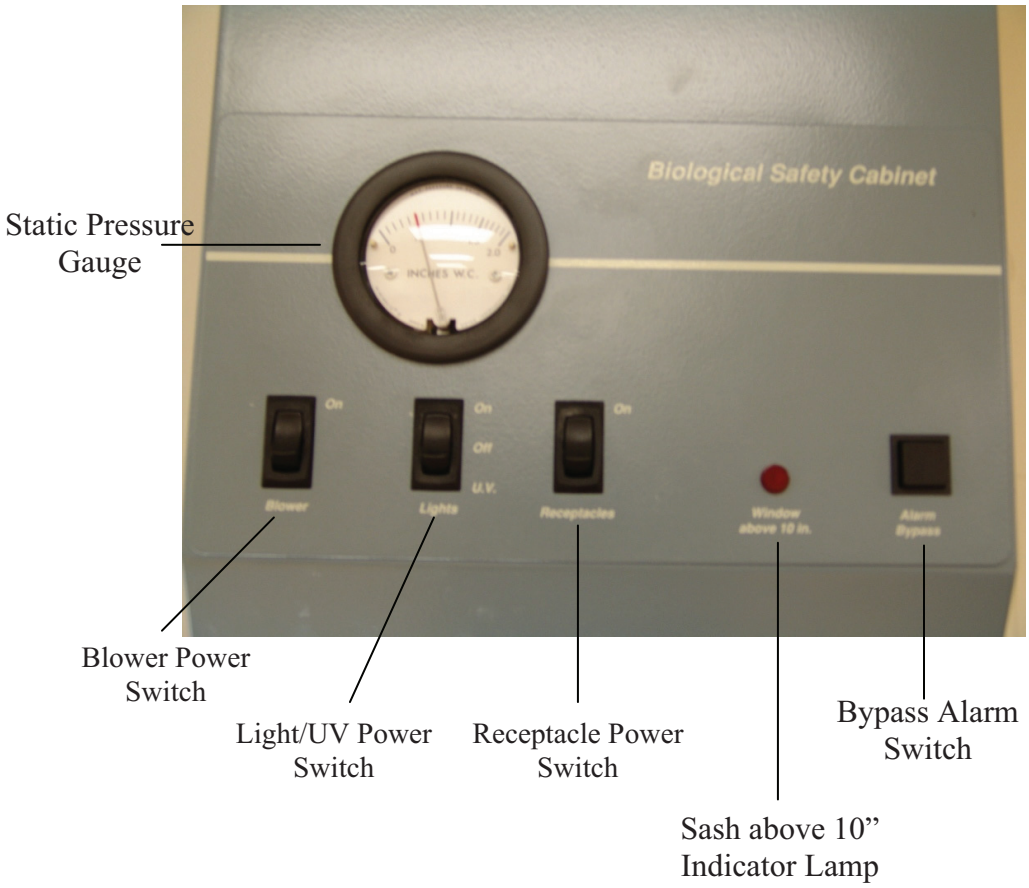

Figure 1: Biological Safety Cabinet Control Panel

Title: Biological Safety Cabinet (BSC) Operation SOP

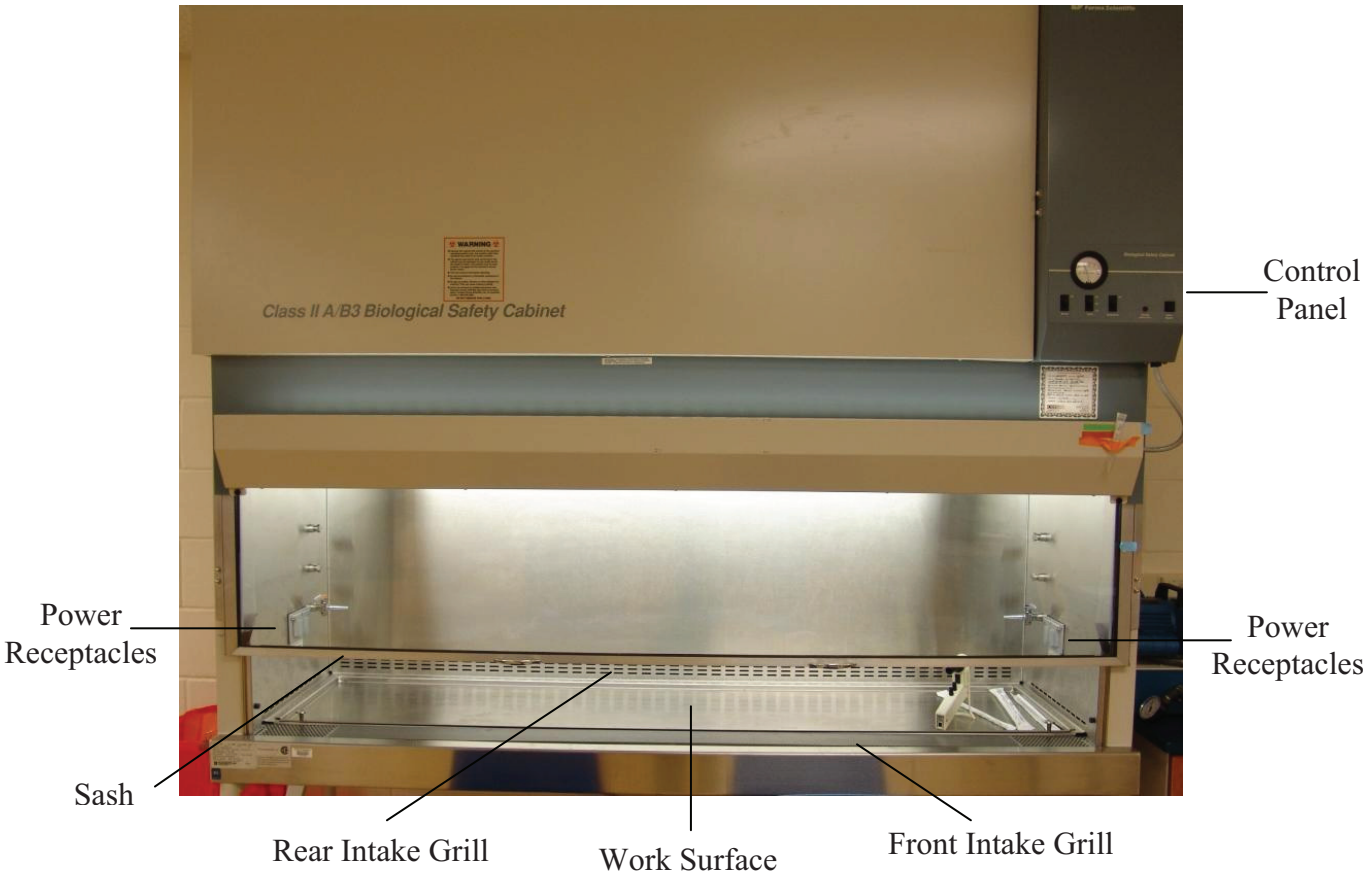

Figure 2: Biological Safety Cabinet Components

Great Bay Community College
320 Corporate Drive
Portsmouth, NH 03801

Bellco Spinner Flask (100mL) Cleaning and Autoclaving SOP

Approvals:

Preparer:_____ Kari Britt _____ Date _____03Apr09_____
Reviewer:_____ Bob O'Brien_____ Date _____03Apr09_____

1. **Purpose:**
   1.1. Cleaning and assembling of the Bellco Spinner Flask (100mL).
2. **Scope:**
   2.1. Applies to the Bellco Spinner Flask (100mL) for maintaining suspension of cultures.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that the SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1.1. Bellco Adjustable Hanging Bar Spinner Flask (100mL) manufacturer insert
   4.1.2. autoclave SOP
   4.1.3. Bellco Micro-Carrier Spinner Flasks (500mL) SOP
   4.1.4. http://www.bellcoglass.com/
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. magnetic stirrer plate
   7.2. glassware detergent
   7.3. bottle brush
   7.4. autoclave
8. **Procedure:**
   8.1. **Preparation**
      8.1.1. Gather all parts: glass spinner flask body, sidearm caps, top cap, compression fitting body, compression fitting retaining nut, cap liner, shaft lock (cap nut, washer and o-ring), glass impeller shaft, magnet and magnet holder.
      8.1.2. Wash all parts with a bottle brush, warm water and glassware detergent. Rinse several times with tap water, and then several times with deionized water.
   8.2. **Assembly**
      8.2.1. Insert the glass impeller shaft into the hole at the bottom of the magnet holder. The knob at the bottom of the shaft will catch and the magnet holder should spin freely.
      8.2.2. Insert magnet into the remaining holes on the magnet holder so that it is centered across the bottom.
      8.2.3. Insert cap liner into the top cap. Push the compression fitting body up through the hole in the bottom of the top cap and lock in place using the retainer nut on the topside of the top cap.
      8.2.4. Gently slide the glass impeller shaft through the cap from the bottom. Slide the o-ring onto the shaft so that it is flush with the cap liner.

Bellco Spinner Flask (100mL) Cleaning and Autoclaving SOP

8.2.5. Insert the impeller assembly into the glass spinner flask body and screw on the top cap.
8.2.6. Adjust the height of the impeller assembly by sliding the glass shaft up or down so that the magnet hangs just above the floor of the glass spinner flask body.
8.2.7. Slide the cap nut onto the glass impeller shaft on top of the washer and tighten.
8.2.8. Test the behavior of the impeller assembly by placing the assembled flask on a magnetic stir plate, and adjusting the height of the glass impeller shaft until the impeller spins properly.
8.2.9. Screw on the side arm caps.
8.3.1. Autoclave per autoclave SOP before and after use. (Remember to loosen side arm caps prior to autoclaving).

9. Attachments:

9.1. Figure 1: Spinner Flask Cap Assembly (http://www.bellcoglass.com/)
9.2. Figure 2: Spinner Flask Impeller Assembly (http://www.bellcoglass.com/)

10. History:

Name	Date	Amendment
Kari Britt Katrice Jalbert	03Feb06	Initial release
Deb Audino	04Apr08	College name change
Kari Britt	03Apr09	Formatting and clarification to directions

Bellco Spinner Flask (100mL) Cleaning and Autoclaving SOP

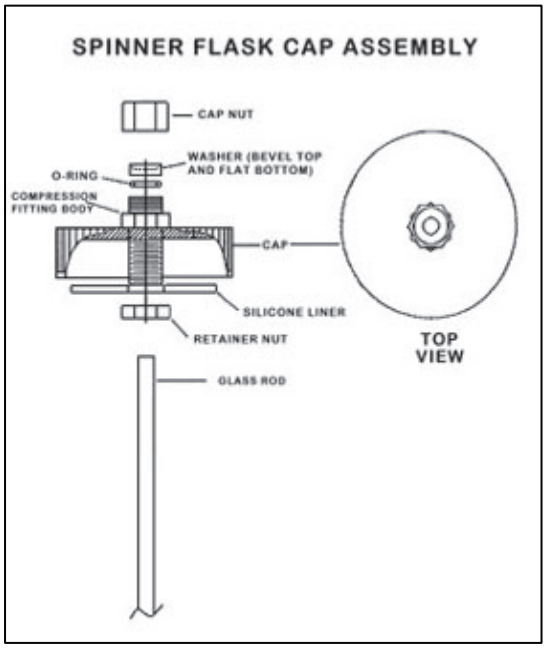

Figure 1: Spinner Flask Cap Assembly
(http://www.bellcoglass.com/)

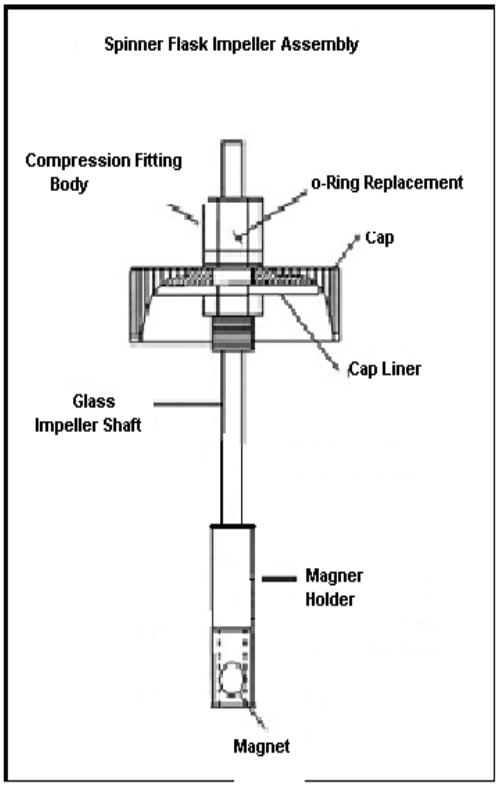

Figure 2: Spinner Flask Impeller Assembly
(http://www.bellcoglass.com/)

Title: Sanyo CO2 Incubator MCO-180IC Operation SOP

Approvals:
Preparer: _Bob O'Brien ___ Date: __17Jul08_____
Reviewer: _Deb Audino_____ Date: __17Jul08_____

1. **Purpose:**
   1.1. Operation of the Sanyo CO2 incubator.
2. **Scope:**
   2.1. Applies to operation of the incubator for mammalian cell culture.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Sanyo instruction manual MCO-18AIC.
5. **Definitions:** N/A
6. **Precautions:**
   6.1. There is a possibility of oxygen depletion in the vicinity of the equipment.
   6.2. Do not lean on the chamber doors.
7. **Materials:**
   7.1. liquefied CO_2 gas bone dry cylinder
   7.2. CO_2 gas regulator
   7.3. tubing
   7.4. tubing clamps
   7.5. biopure water (filtered deionized water)
   7.6. 70% isopropanol (IPA)
8. **Procedure:**
   Note: Refer to Figures 1 and 2 as needed throughout this procedure.
   8.1. **Setup**
      8.1.1. Turn power on (if necessary).
      8.1.2. The current incubator temperature and CO_2 level will be displayed on the control panel.
   8.2. **Set the temperature.**
      8.2.1. Press the Set key on the control panel.
      8.2.2. The left most temperature digit will automatically flash.
      8.2.3. Press the Numerical Value Shift key ▲ ▲ to change the numerical value to the desired temperature.
      8.2.4. Press the Digit Shift key ►► to shift digit positions to the middle position. The temperature digit will automatically flash.
      8.2.5. Press the Numerical Value Shift key ▲ ▲ to change the numerical value to the desired temperature.
      8.2.6. Press the Digit Shift key ►► to shift digit positions to the right most position.

Title: Sanyo CO2 Incubator MCO-180IC Operation SOP

8.2.7. Press the Numerical Value Shift key ▲▲ to change the numerical value to the desired temperature.
8.2.8. Press the "ENT" key to allow the controller to accept the input temperature.

8.3. **Set up the CO_2 feed.**
8.3.1. Verify that the tubing clamp is open to allow CO_2 gas into the feed supply for the incubator.
8.3.2. Verify that the open/close valve on the CO_2 cylinder is in the open position and that the CO2 regulator is turned on to the required pressure.
8.3.3. Press the "Set" key to set CO2.
8.3.4. The left most temperature digit will automatically flash, and the left most carbon dioxide digit will automatically flash.
8.3.5. Press the Numerical Value shift key ▲▲ on the control panel until the display shows the desired operating CO_2 level.
8.3.6. Press the Digit Shift key ►►to shift digit positions and the Numerical Value Shift key ▲▲ to change the numerical value.
8.3.7. Press the enter key twice on the control panel to enter the percentage of CO_2 into the memory.
8.3.8. When the CO_2 density is set to 0.00, the control is off regardless of the chamber density.

8.4. **Operation**
8.4.1. Verify that the actual temperature and percentage of CO_2 are at the desired levels.
8.4.2. Clean the outer surface of the vessel containing the culture or sample to be incubated with 70% IPA prior to placing it in the incubator.
8.4.3. Open the outer incubator chamber door.
8.4.4. Open the inner incubator chamber door.
8.4.5. Place the culture to be incubated in the center of the tray if possible.
8.4.6. Close the inner incubator chamber door.
8.4.7. Close the outer incubator chamber door.
8.4.8. Verify that the operating temperature and CO_2 levels on the control panel are set to the recommended levels for the culture or sample being incubated.

9. Attachments:
9.1. Figure 1: Incubator Components
9.2. Figure 2: Control Panel

10. History:

Name	Date	Amendment
Bob O'Brien	03Feb05	Initial Release
Bob O'Brien	13Apr07	Updated the date format. Added key symbols to directions as needed.
Bob O'Brien	18Jul08	College name change

Title: Sanyo CO2 Incubator MCO-180IC Operation SOP

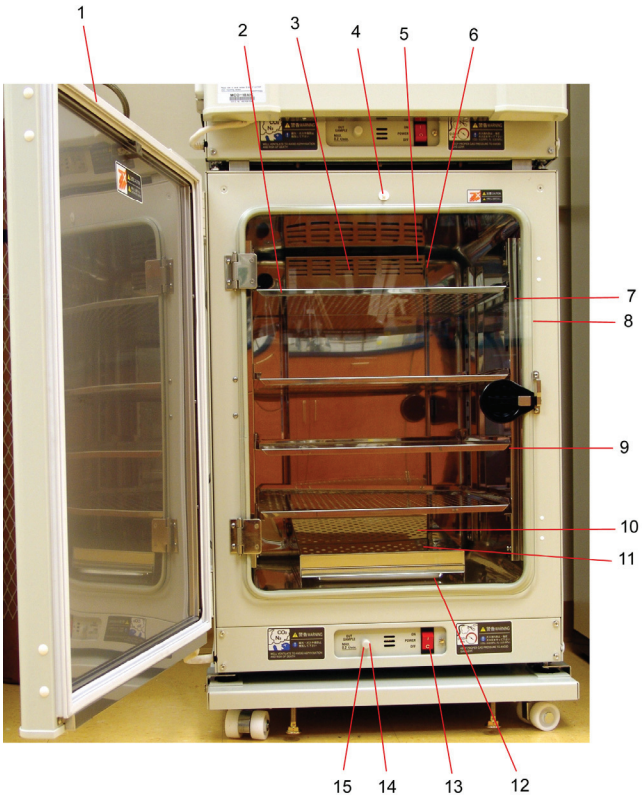

Figure 1: Incubator Components

1. **Outer Door:** sticks to frame with magnetic seal; door heater installed in the door panel
2. **Tray Support:** can be removed by lifting the front side and pulling it toward the door
3. **Top Duct:** inlet area for circulating air
4. **Door Switch:** detects door position (open/closed); stops the fan and CO_2 gas feed
5. **Circulation Fan:** located inside the rear duct; fan can be autoclaved
6. **Rear Duct:** flow path for circulating air output; removable for access to circulation
7. **Side Support:** right and left supports can be removed for cleaning or disinfecting
8. **Inner Door:** made of tempered glass, avoid excessive impact on the glass
9. **Tray:** structure for support of samples and stir plates.
10. **Water Level Sensor:** detects the water level in the humidifying pan; located under the humidifying pan cover
11. **Humidifying Pan Cover:** prevents spills from contaminating the humidifying pan
12. **Humidifying Pan:** properly installed when resting on the floor of the incubator cabinet and covered by the humidifying pan cover; only use biopure water to fill the pan
13. **Power Switch:** this is the main power switch for the incubator
14. **Air Sampler Outlet Cap:** always attached except at time of air sampling
15. **Air Sampler Outlet:** use this outlet for air sampling; this also functions as an internal gas outlet; should be covered with cap

Title: Sanyo CO2 Incubator MCO-180IC Operation SOP

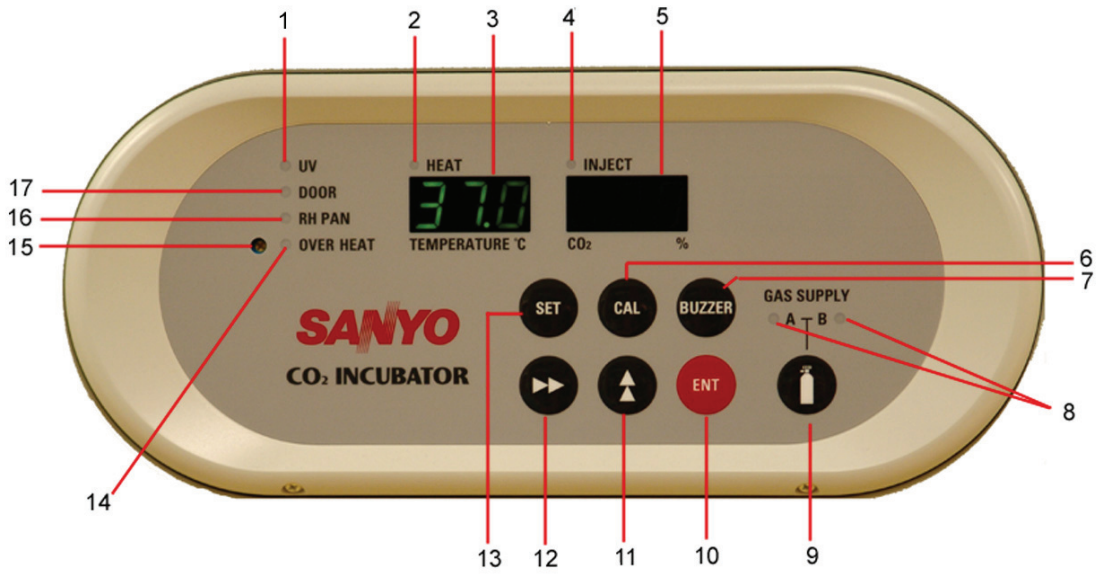

Figure 2: Control Panel

1. **UV Indicator** (this model is not equipped with UV)
2. **Heat Indicator:** this light comes on when the heater is energized
3. **Digital Temperature Indicator:** indicates current chamber operating temperature
4. **CO_2 Inject Indicator:** this light comes on when CO_2 gas is being injected
5. **Digital CO_2 Density Indicator:** indicates current percentage of CO_2 in the chamber
6. **Calibration Key:** not necessary to touch
7. **Alarm Buzzer:** press the "buzzer" key to silence the alarm when needed
8. **CO_2 Gas Supply Line Indicator** (not installed in this model).
9. **CO_2 Gas Supply Line Switching Key** (not installed in this model).
10. **Enter Key:** pressing this key inputs the set value into the controller
11. **Numerical Value Shift Key ▲▲ :** press this key in the set mode to change the numerical value
12. **Digit Shift Key ►►:** Press this key in the set mode to shift the position of the digit
13. **Set Key:** Press this key to access to the set mode
14. **Over-heat Indicator:** this light comes on when the chamber temperature reaches the upper limit set value
15. **Upper Limit Regulator:** This regulator is used to set the upper temperature limit
16. **RH Pan Indicator:** this flashes when water in the humidifying pan is less than ~1 liter
17. **Door Indicator:** this light comes on when the door is open

Title: Applikon Bioreactor Operation SOP

Approvals:
Preparer: ____Bob O'Brien_____Date _____03Apr08_____
Reviewer: ___Deb Audino_____ Date _____03Apr08_____

1. **Purpose:**
   1.1. Operation of the Applikon 3L Benchtop Bioreactor.
2. **Scope:**
   2.1. Applies to growing mammalian or insect cells in the Applikon 3L Benchtop Bioreactor.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Applikon Benchtop Bioreactor manufacturer instrumentation manuals
   4.2. autoclave SOP
   4.3. biological safety cabinet SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. The sodium bicarbonate solution is very basic/caustic. Prevent contact with skin.
   6.2. The O_2 electrolyte used in the DO probe is a strong Alkaline (pH13) solution. Avoid contact with skin, mucous membrane, or eyes. If contact does occur flush effected area with water.
   6.3. The heat blanket is very warm when in use. Use caution.
7. **Materials :**
   7.1. pH 4.0 buffer standard
   7.2. pH 7.0 buffer standard
   7.3. lab towels
   7.4. 1X PBS
   7.5. 1M sodium bicarbonate
   7.6. silcone tubing
   7.7. PharMed tubing
   7.8. clamps
   7.9. aluminum foil
   7.10. air filters (4)
   7.11. glass wool
   7.12. probe stand with clamps
   7.13. 15mL conical tube
   7.14. O_2 electrolyte (for DO probe)
   7.15. 3M potassium chloride (KCL) solution for pH probe storage

Title: Applikon Bioreactor Operation SOP

7.16. Bioreactor
- 7.16.1. glass pH probe
- 7.16.2. stainless steel DO probe
- 7.16.3. Pt-100 temperature probe
- 7.16.4. harvest tube
- 7.16.5. sparger
- 7.16.6. thermowell tube
- 7.16.7. glass vessel
- 7.16.8. condenser
- 7.16.9. feed bottle
- 7.16.10. sample bottle assembly
- 7.16.11. heater blanket

7.17. autoclave
7.18. biological safety cabinet
7.19. glycerol
7.20. transfer pipets
7.21. source of CO_2
7.22. source of air
7.23. 1L sterile bottles
7.24. 500mL sterile bottles

8. Procedure:

8.1. **Assemble Vessel Stand (Figure 1)**
- 8.1.1. Inspect the integrity of the large O-rings on the vessel stand and headplate.
- 8.1.2. Replace if worn or cracked.
- 8.1.3. Lower glass vessel into the vessel stand, making contact with the O-ring.
- 8.1.4. Verify that the bolts are threaded completely into the vessel stand. Thread if necessary.
- 8.1.5. If preparing for a run, add approximately 25mL 1X PBS to the vessel and approximately 250mL of 1M Sodium bicarbonate to the feed bottle.

8.2. **Assemble Headplate –Underside (Figure 2)**
- 8.2.1. Inspect the integrity of the O-rings on the harvest tube, sparger tube, and thermowell tube. Replace if worn or cracked.
- 8.2.2. Put two inch long piece of tubing on the non-barbed end of the harvest tube.
- 8.2.3. Insert the harvest tube from the underside of the headplate between the inoculation port and 3 addition port.
- 8.2.4. Place a washer and nut onto the harvest tube on the top of the headplate. Tighten the nut until the retainer of the harvest tube is flush with the underside of the headplate.
- 8.2.5. Insert the sparger tube from the underside of the headplate, next to blind stopper.
- 8.2.6. Rotate sparger tube so it is aligned beneath the stirrer impeller.

Title: Applikon Bioreactor Operation SOP

8.2.7. Place a washer and nut onto the sparger tube on the top of the headplate. Tighten the nut until the retainer of the sparger tube is flush with the underside of the headplate.
8.2.8. Insert the thermowell tube from the underside of the headplate, in between the pH and DO probe ports.
8.2.9. Place a washer and nut onto the thermowell tube on the top of the headplate. Tighten the nut until the retainer of the thermowell tube is flush with the underside of the headplate.

8.3. **Attach Headplate to Vessel Stand (Figure 3)**
8.3.1. Place the headplate onto the vessel stand, positioning the holes on the outer edge of the headplate with the bolts on the vessel stand.
8.3.2. Place the sample bottle assembly onto the bolt located by the 3 addition port and attach with a mill fastener.
8.3.3. Secure the headplate with the remaining 5 mill fasteners by hand in a crosswise pattern.

8.4. **Assemble Headplate – Top Side (Figure 3)**
8.4.1. Inspect the integrity of the O-ring in the condenser port of the headplate. Replace if worn or cracked.
8.4.2. Inspect the black seal at the bottom of the condenser underneath the retainer nut. Replace if worn or cracked.
8.4.3. Place the condenser into the condenser port making sure that the barbed connectors are facing out.
8.4.4. Tighten the retainer nut.
8.4.5. **DO Probe**
8.4.5.1. Remove the protective cap from the bottom of the stainless steel DO probe.
8.4.5.2. Inspect the screen at the bottom of the probe tip. Replace if damaged.
8.4.5.3. Holding the probe in a vertical position, unscrew the membrane module from the bottom of the probe.
8.4.5.4. Inspect the integrity of the O-ring underneath the module and replace if worn or cracked.
8.4.5.5. Replenish DO electrolyte. There should be approximately 1mL of 0_2 electrolyte solution in the membrane module.
8.4.5.6. Replace membrane module.
8.4.5.7. Inspect the integrity of the O-ring at the top of the stainless steel DO probe. Replace if worn or cracked.
8.4.5.8. Inspect the black seal at the top of the DO probe under the retainer nut. Replace if worn or cracked.
8.4.5.9. Place the DO probe into the DO probe port and tighten retainer nut.

8.5. **pH Probe Calibration**
8.5.1. Remove the protective caps from the top and bottom of the glass pH probe.
8.5.2. Pour the 3M potassium chloride (KCL) storage solution from the bottom protective cap into the 15mL conical tube.
8.5.3. Connect the pH Controller cable to the top of the pH probe.

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8.5.4. Turn on the ADI1025 unit by pressing the large green button on the front of the unit.
8.5.5. Wait for instrument to initialize (approximately one minute).
8.5.6. The set point main screen will appear on the display screen.
8.5.7. On the ADI1010, press the "pH" key then the "Calib." key.
8.5.8. Use the dial on the ADI1010 to highlight "reset to default calibration values."
8.5.9. Press the "Calib." key to input the factory defaults into the ADI1010 controller.
8.5.10. Press the "pH" key then the "Calib." key.
8.5.11. Use the dial on the ADI1010 to highlight the calibration option.
8.5.12. Press the "Calib." key to select the calibration option. This will display the pH calibration screen.
8.5.13. Rinse the pH and Pt-100 temperature probes with DI water and blot dry.
8.5.14. Place the pH and Pt-100 temperature probes into the pH 7 buffer standard.
8.5.15. Allow the pH and temperature readings to stabilize.
8.5.16. Press "Start/Stop" key to input the temperature value.
8.5.17. Press the "Calib." key.
8.5.18. Use the dial on the ADI1010 to input the pH value of the standard (7.00).
8.5.19. Press the "Calib." key.
8.5.20. Rinse the pH and Pt-100 probe with DI water and blot dry.
8.5.21. Place the pH and Pt-100 probe in to the pH 4 buffer standard.
8.5.22. Allow the pH reading to stabilize.
8.5.23. Use the dial on the ADI1010 to input the pH value of the standard (4.00).
8.5.24. Press the "Calib." Key. This will display the pH calibration slope and offset values.
8.5.24.1. Expected values:
Slope: 0.95-1.05
Offset: $< \pm 0.3$
8.5.24.2. See supervisor if not within this range.
8.5.25. Press the "Setp." key twice to return to the main screen.
8.5.26. Turn off power by pressing the large green button on the ADI1025 unit.
8.5.27. Remove both probes from pH 4 buffer standard.
8.5.28. Rinse both probes with DI water and blot dry.
8.5.29. Disconnect the pH probe from the controller cable and replace the top protective cap.
8.5.30. Inspect the integrity of the O-ring at the top of the glass pH probe. Replace if worn or cracked.
8.5.31. Inspect the black seal at the top of the pH probe under the retainer nut. Replace if worn or cracked.
8.5.32. Place the pH probe into the pH probe port and tighten the retainer nut.

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8.6. **Attach Tubing/ Autoclave Preparation**

8.6.1. Connect labeled end of silicone tubing with air filters on the following :
sparger tube
condenser top outlet
CO_2 overlay port

8.6.2. Use a small piece of silicone tubing to connect together two of the ports on the 3 addition port.

8.6.3. Connect the PharMed tubing from the feed bottle to the 3 addition port.

8.6.4. Connect the sample bottle tubing to the harvest tube.

8.6.5. Clamp off all tubing (near the headplate) except the condenser top outlet. The condenser top outlet must remain unclamped to release pressure during autoclaving.

8.6.6. Close all open ends with glass wool and cover with aluminum foil.

8.7. **Autoclave**

8.7.1. Prepare autoclave per autoclave SOP.

8.7.2. Transfer bioreactor vessel to autoclave.

8.7.3. Autoclave at 121°C for 20 minutes, using slow exhaust.

8.7.4. Remove from autoclave when cycle is complete and return to bench.

8.8. **Polarize DO Probe**

8.8.1. Remove protective caps from the pH and DO probes.

8.8.2. Connect the pH and DO probes to the appropriate power cable.

8.8.3. Wrap the thermal blanket around the vessel and plug into the ADI1025.

8.8.4. Turn on power by pressing the large green button on the ADI1025 unit.

8.8.5. Allow DO probe to polarize for at least 6 hours before performing DO calibration.

8.9. **Media Addition**

8.9.1. Aseptically add media (per the process SOP) to the bioreactor vessel.

8.10. **Run Preparation**

8.10.1. Place stirrer motor on stirrer motor mount.

8.10.2. Remove the clamps on the following tubing:
feed bottle
CO_2 overlay port
sparger

8.10.3. Place tubing from the feed bottle through the feed pump on the ADI1025.

8.10.4. Connect the tubing from the CO_2 overlay port to the overlay outlet on the ADI1025.

8.10.5. Connect the tubing from the sparger to the sparger outlet on the ADI1025.

8.10.6. Using a transfer pipet, add a small amount (1.5-2mL) of glycerol to the thermowell.

8.10.7. Place the Pt-100 temperature probe into the thermowell.

8.11. **Establish Set Points**

8.11.1. On the ADI1010, press a parameter key (pH, Temp, dO_2, or Stirrer) then the "Setp." key.

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8.11.2. Use the dial to set the desired set point per the process SOP.
8.11.3. Press the "Setp." key to return to the Main Menu.
8.11.4. Repeat until each set point in the process SOP is established per the process SOP.

8.12. **Establish Limits**
8.12.1. On the ADI1010, press a parameter key.
8.12.2. Press the "limits" key to go to the upper limit.
8.12.3. Use the dial to set the upper limit per the process SOP.
8.12.4. Press the 'limits" key again to go to the lower limit.
8.12.5. Use the dial to set the lower limit per the process SOP.
8.12.6. Press the "Setp." key to return to the Main Menu
8.12.7. Repeat until an upper and lower limit is set for each parameter per the process SOP.

8.13. **Activate Control Loops**
8.13.1. Press the pH key then the "Start/Stop" key to activate the pH control loop.
8.13.2. Press the "Temp" key then the "Start/Stop" key to activate the temperature control loop.
8.13.3. Press the "Stirrer" key then the "Start/Stop" key to activate the stirrer control loop.
8.13.4. Lights on the pH, temperature, and stirrer keys should turn green.
8.13.5. NOTE: Do NOT activate the dO_2 control loop at this time.

8.14. **DO Probe Calibration (after six hours of polarization)**
8.14.1. On the ADI1010, press the "dO_2" key then the "Calib." key.
8.14.2. Four options are displayed: (View Calibration Values, Temperature Compensation, Calibration, and Reset to Default Calibration Values.)
8.14.3. Use the dial on the ADI1010 to highlight the calibration option.
8.14.4. Press the "Calib." key to select the calibration option.
8.14.5. Use the dial on the ADI1010 to set the value to100.
8.14.6. Press the "Calib." key.
8.14.7. The DO probe calibration slope should be on the ADI1010 display.
8.14.7.1. Expected values are:
Slope: 8-15 at 37^{o}C or 10-20 at 25^{o}C
8.14.7.2. See supervisor if not within this range.
8.14.8. Press the "Setp." key to return to the main screen on the ADI1010 display.
8.14.9. Press the "dO_2" key then the "Start/Stop" key to activate the dO_2 control loop.
8.14.10. Lights on the "dO_2" key should turn green.
8.14.11. Disconnect the DO probe BRIEFLY (<1min) from the DO cable.
8.14.11.1. The DO reading should quickly decrease to 0.
8.14.11.2. If the DO value does not drop below 0.5% the probe requires maintenance (refer to the user manual for the DO probe).
8.14.11.3. Reconnect the DO cable to the DO probe.
8.14.12. Turn on Air and CO_2 supplies to the bioreactor.

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8.15. **Add cell culture through the Inoculation port per the process SOP.**

8.16. **BioXpert Lite**

- 8.16.1. Turn on power to computer.
- 8.16.2. Open BioXpert Lite software.
- 8.16.3. Choose: Username: NHCTC, Mode: Cultivation, and Fermentor no.:1.
- 8.16.4. Select "OK".
- 8.16.5. To ensure the computer is communicating with the controller, on the menu bar, click on Run, Test. Next to each variable, a number should be displayed in the values column representing the value of that parameter.
  - 8.16.5.1. If no numbers are displayed, notify your instructor.
- 8.16.6. Select "OK".
- 8.16.7. On the menu bar, click on Run, New.
- 8.16.8. In the Organism box, type in the name of the cells being grown. Type any comments in the Comments box.
- 8.16.9. Select "OK".
- 8.16.10. Choose the access interval between readings.
  - 8.16.10.1. This can be changed during a run without changing the start moment.
- 8.16.11. Under Protocol in the Data box type "F" (for fall) or "S" (for spring) and the year (i.e. F2006).
  - 8.16.11.1. Note: The Data box allows a maximum of 8 characters.
- 8.16.12. Select "OK".
  - 8.16.12.1. Note: Selecting "OK" begins the data collecting process.
- 8.16.13. **Important:** Do NOT close (i.e. "X-out of) the On-line Session window or select "END" at **any** time during the run. Doing this will cause the **loss** of **all** data for the run.
- 8.16.14. To view the Chart window during a run, simply click on the blue bar at the top of the Chart window. This will bring the chart to the front. Do the same to view the On-line Session window if it is behind the Chart window.

8.17. **Setting the scale of the graph**

- 8.17.1. View the Chart window in the BioXpert light application by clicking on the blue bar at the top of the chart window.
- 8.17.2. View the "Y" axis variables by clicking on a parameter in the Variables window. All of the variables can be viewed at once.
- 8.17.3. Use the mouse to hold the curser over any of the Y axis variable lines (pH, Temp, dO_2, or Stirrer) in the chart. Left click to open the Scale Settings window.
- 8.17.4. Check the box next to "Manually Scale".
- 8.17.5. Choose the Y axis you would like to change in the "List of Variables" pull-down menu.
- 8.17.6. Change the scale by entering the desired values in the Scale range boxes.
- 8.17.7. Select "OK".

8.18. **Ending a Run**

- 8.18.1. On the menu bar, click on File, Save as. Delete the (*) and enter a file name.

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8.18.2. Select "OK".
8.18.3. Export the data to EXCEL by choosing File, Export, Data on the menu bar.
8.18.3.1. Select the following options for export:
Format: SYLK (for EXCEL)
Variables: On-lines (Off-lines should not be checked.)
Time Interval: On-line
Time Range: All
8.18.3.2. Note: In the Time Interval option, On-line will export all data collected at the interval period chosen in section 8.16.10 while Off-line will export data points that were collected every 15 minutes. "Other" is used to export a custom interval**.**
8.18.3.3. Select "OK".
8.18.3.4. Under File Name delete the (*) and type a file name. Make note of the directory where the file is saved before selecting "OK".
8.18.3.5. Select "OK".
8.18.4. Turn off the control loops on the ADI1010by selecting a parameter key ("pH", "Temp", "dO_2", or "Stirrer"), and pressing the "Start /Stop" key. Repeat for each control parameter.
8.18.5. Turn off the Air and CO_2 supplies to the ADI1025 controller.
8.18.6. Aseptically remove cultivation through the harvest port, utilizing the sample bottle.
8.18.7. Turn off power switch on the ADI1025.
8.18.8. Remove stirrer motor.
8.18.9. Unplug the heater blanket and remove from the vessel. Store flat.
8.18.10. Disconnect the power cords for the pH and DO probes.
8.18.11. Remove the pH and DO probes and place in storage clamps.
8.18.12. Place protective caps on the pH and DO probes.
8.18.13. Remove the Pt-100 probe, rinse with DI water and blot dry.
8.18.14. Remove all tubing.

8.19. **Cleaning the Bioreactor**
8.19.1. Clean pH and DO probes.
8.19.1.1. Remove pH and DO probes from the headplate.
8.19.1.2. Wipe down DO and pH probes with a lab towel damp with 10% bleach solution.
8.19.1.3. Rinse DO and pH probes with DI water and blot dry.
8.19.2. Remove the headplate from the vessel (with harvest tube, thermowell tube and sparger tube still attached.
8.19.3. Add 1L of 10% bleach solution to the vessel.
8.19.4. Replace the headplate and soak the sparger tube, harvest tube and thermowell tube for approximately 15 minutes.
8.19.5. Remove headplate from vessel.

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8.19.6. Disassemble the entire headplate assembly and wash all of the components with 10% bleach solution. Rinse with DI water, and spray with 70% IPA. Set out on lab towels to air dry.
8.19.7. Clean all tubing with 10% bleach solution, rinse with DI water, and spray with 70% IPA. Set out on lab towels to air dry.
8.19.8. Wipe down controller with a lab towel damp with a 10% bleach solution, then with a towel damp with DI water.
8.19.9. Place headplate and components out on lab towels to dry.
8.19.10. After the components have dried, reassemble the bioreactor for storage (leaving out the pH and DO probes).

8.20. **pH probe Storage**
8.20.1. After cleaning the pH probe, verify that no broth or media residue remains on the membrane surface or diaphragm.
8.20.2. Pour the 3M potassium chloride (KCl) solution from the 15mL conical tube into the protective cap. Add more 3M potassium chloride (KCl) solution if necessary to fill the cap ½ full with the solution.
8.20.3. Insert the electrode end of the pH probe into the protective cap. The electrode should be completely immersed in the 3M potassium chloride (KCl).
8.20.4. Place the probe in a storage clamp and store in vertical position.

8.21. **DO probe Storage**
8.21.1. After cleaning the DO probe, verify that no broth or media residue remains on the membrane surface or diaphragm.
8.21.2. Holding the probe in a vertical position, unscrew the membrane module from the bottom of the probe and verify that approximately 1mL of O_2 electrolyte solution is present in the membrane module.
8.21.3. Replace the protective cap. Place probe in storage clamp, and store in vertical position.

9. Attachments:
9.1. Figure 1: Vessel and Headplate
9.2. Figure 2: Underside of the Headplate
9.3. Figure 3: Assembled Headplate
9.4. Figure 4: ADI1010 Controller

10. History:

Name	Date	Amendment
Bob O'Brien Deb Audino	072705	Initial Release
Deb Audino Bob O'Brien	100605	Deb Audino and Bob O'Brien, 100605, Removed all grease steps, reduced amount of PBS to add to vessel, removed PBS removal step, modified adding media in BSC, modified cleaning steps.
Bob O'Brien	042706	Add O_2 in precaution, additional materials for probe storage, modify section 8.5 for calibration. Add section 8.20 pH probe storage; add section 8.21 DO probe storage.
Kari Britt	09Oct06	New directions for BioXpert Lite section to correspond with

Title: Applikon Bioreactor Operation SOP

		upgrade to new version to computers. Moved Export the Data section to the Ending a run section in order to consolidate BioXpert Lite directions.
Bob O'Brien	04Apr08	College name change

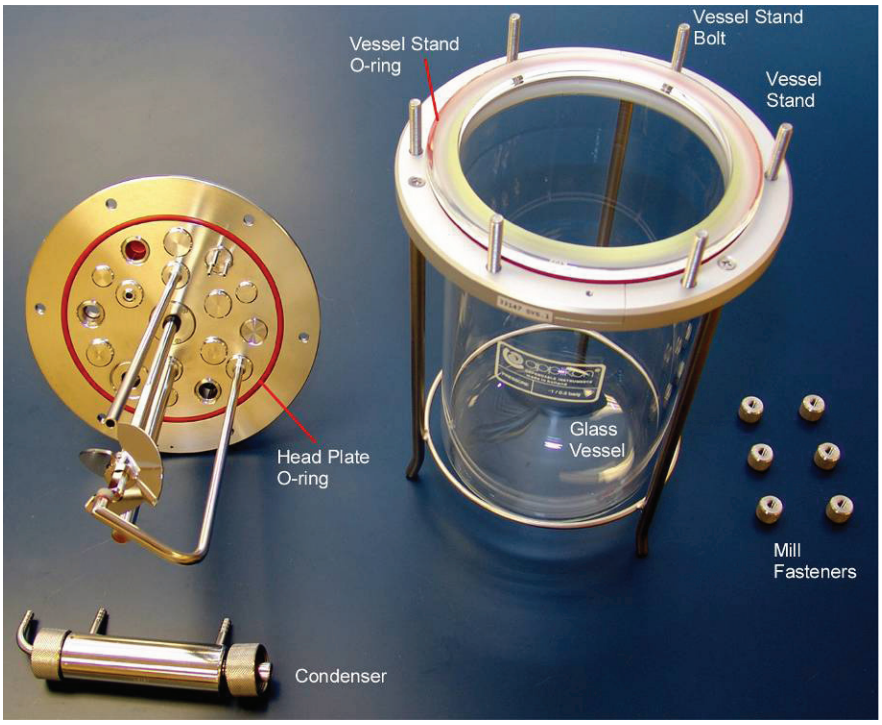

Figure 1: Vessel and Headplate

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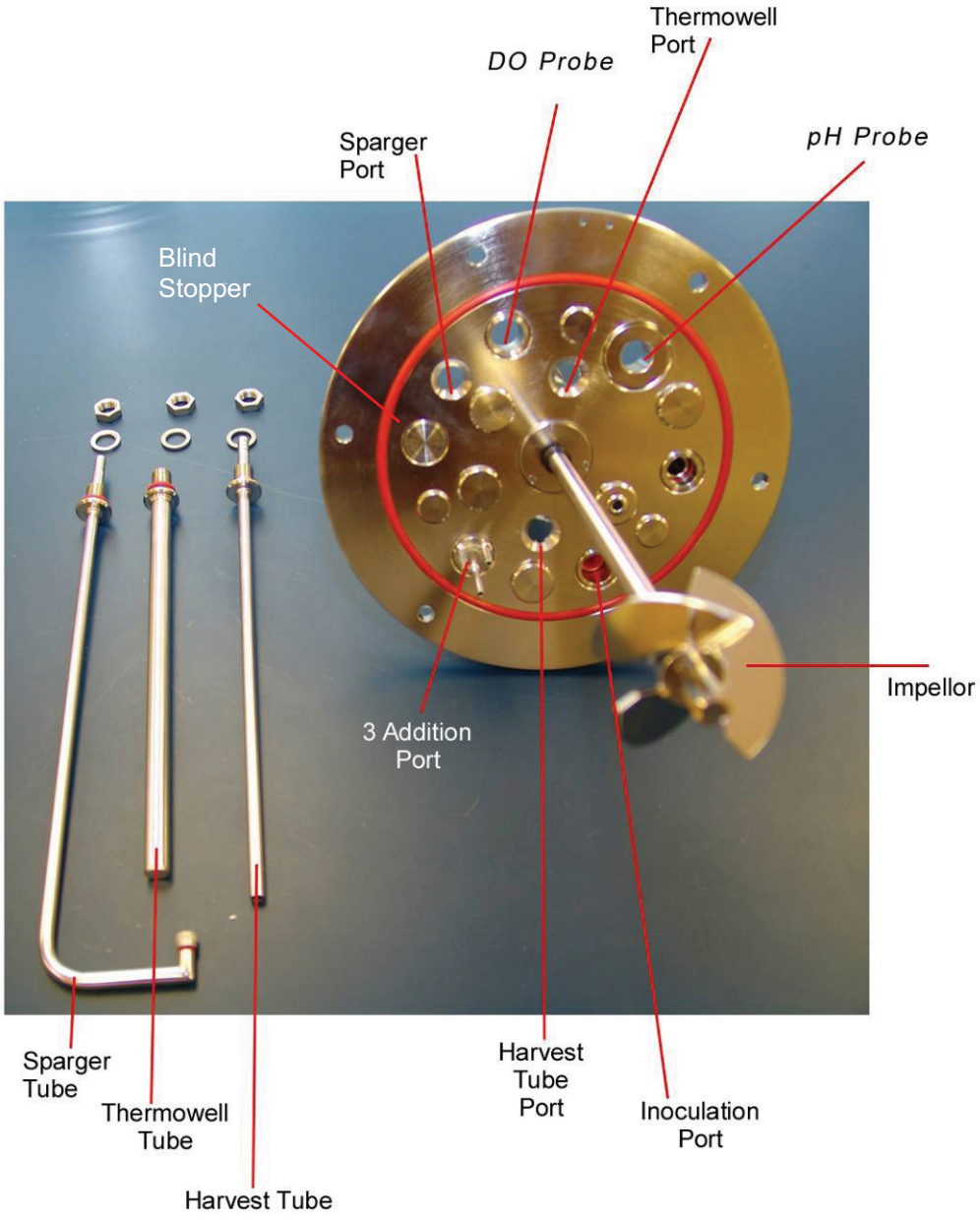

Figure 2: Underside of Headplate

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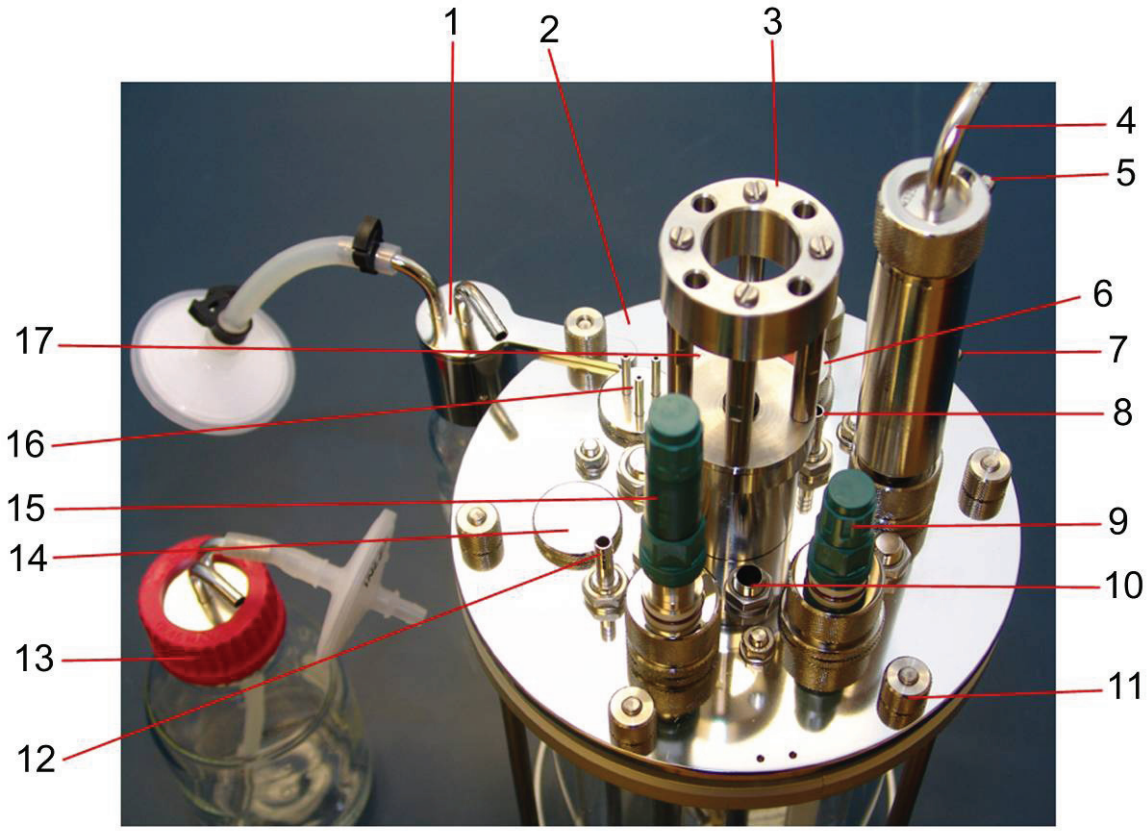

Figure 3: Assembled Headplate

1. Sample bottle assembly
2. Head plate assembly
3. Stirrer motor mount
4. Condenser air outlet
5. Condenser water outlet (from)
6. Inoculation port
7. Condenser water inlet (to)
8. CO_2 overlay port
9. pH probe
10. Thermowell port
11. Mill fastener
12. Sparger
13. Feed bottle
14. Blind stopper
15. DO probe
16. 3 Addition port
17. Harvest tube

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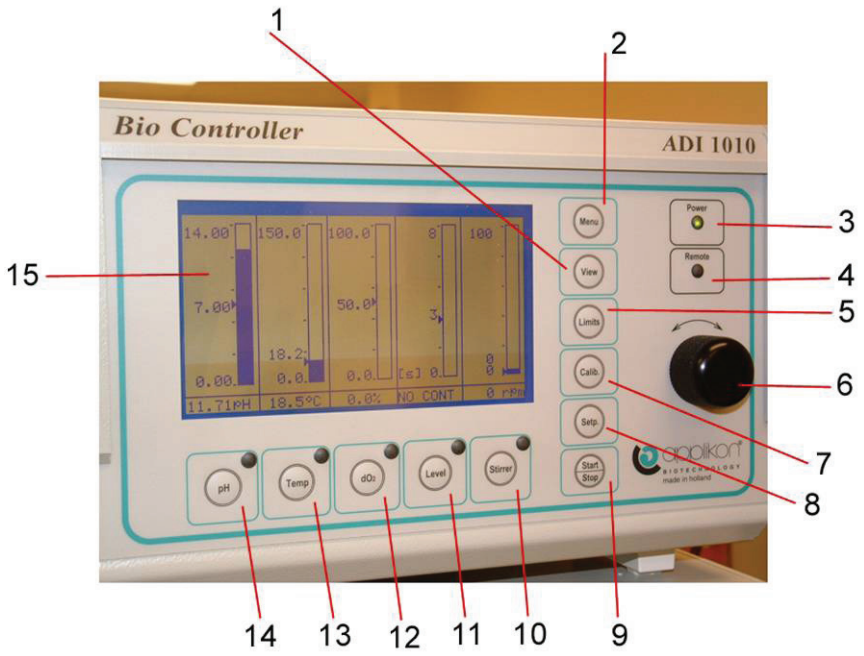


Figure 4: ADI1010 Controller

1. View function key.
2. Menu function key
3. Power indicator LED
4. Remote indicator LED
5. Limits function key
6. Dial: digital potentiometer
7. Calib. Key
8. Setup key
9. Start/Stop key
10. Stirrer parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, and alternating Green/Red out of range
11. Level parameter key; has a dual color LED, off, Green on no alarm, Red out of range, and alternating Green/Red out of range
12. DO parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, alternating Green/Red out of range
13. Temp parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, alternating Green/Red out of range
14. pH parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, alternating Green/Red out of range
15. Display screen

Great Bay Community College
320 Corporate Drive
Portsmouth, NH 03801

Title: Trypan Blue Assay SOP

Approvals:
Preparer:_____Kari Britt _____ Date _____05Aug10_____
Reviewer:_____Sonia Wallman_____ Date _____05Aug10_____

1. **Purpose:**
   1.1. Use of the Trypan Blue Assay.
2. **Scope:**
   2.1. Applies to determining viable cell count of mammalian and insect cells.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. http://plaza.ufl.edu/johnaris/Protocols/MiscMethods/HemoCytometer.pdf
   4.2. microscope SOP
5. **Definitions:**
   5.1. Hemacytometer: a specialized microscope slide with etched glass in grid formation
6. **Precautions:**
   6.1. Trypan Blue Solution is a teratogen. It may cause birth defects. It may cause cancer. Wear gloves, eye protection and a lab coat.
7. **Materials:**
   7.1. 0.4% Trypan Blue Solution
   7.2. microfuge tubes
   7.3. P-20 micropipette and tips
   7.4. cell sample in solution
   7.5. hemacytometer
   7.6. hemacytometer coverslip
   7.7. microscope
   7.8. lab towels
   7.9. lab tissues such as Kimwipes
   7.10. deionized water
   7.11. push button counter
8. **Procedure:**
   8.1. **Mix Trypan Blue Solution with cell sample solution**
      8.1.1. Mix culture sample well to resuspend cells.
      8.1.2. Remove 20µL of culture sample and dispense into a microfuge tube.
      8.1.3. Add 20µLof 0.4% Trypan Blue Solution to the same tube.
      8.1.4. Mix the above solution by gently aspirating and dispensing the solution with the micropipette. Proceed to the next step immediately.
   8.2. **Transfer sample to hemacytometer**
      8.2.1. Center the coverslip on top of the hemacytometer. The metal notches should be partially exposed.

Title: Trypan Blue Assay SOP

8.2.2. Hold the micropipette straight up and dispense 10µL of the cell/Trypan Blue solution into a notch of the hemacytometer. The tip of the pipette should be very close to the metal surface. The solution will spread through capillary action.

8.3. **Observe cells under the microscope**

8.3.1. Turn on the microscope per SOP.

8.3.2. Place the hemacytometer on the microscope stage.

8.3.3. Focus on the hemacytometer grid using 100X magnification (10X objective lens). Live cells are clear. Dead cells are blue.

8.4. **Count cells**

8.4.1. The grid is divided into four main quadrants (Figure 2). Beginning with quadrant 1 and moving through to quadrant 4, depress the correct button on the push button counter for every cell in each square.

8.4.1.1. Left button is for live cells. Right button is for dead cells. Make sure the counter is set to 0.

8.4.1.2. Count in a serpentine fashion: work left to right across the top row of the quadrant. Move down to the second row and count the cells in each square moving right to left. Change to opposite direction each time a row is completed.

8.4.1.3. Count cells touching the top and left borders of a main quadrant, but not the bottom and right borders. **Do not count cells outside of the main quadrants**.

8.4.1.4. Record the number of live and dead cells each time a quadrant is completed.

8.5. **Clean the hemacytometer**

8.5.1. Remove the coverslip.

8.5.2. Blot dry the coverslip and hemacytometer on a lab towel.

8.5.3. Rinse the cover slip and hemacytometer with deionized (DI) water by holding each one over a lab towel and using a squirt bottle of DI water.
Note: Handle the hemacytometer and coverslip gently. The coverslip is not disposable. Do not discard it.

8.5.4. Dry the coverslip and hemacytometer with a lab tissue.

8.6. **Calculate viable cell concentration.**

8.6.1. Formula to determine live cell count: C = (N/V) x D
C = live cell count in cells per milliliter
N = total number of live cells counted in the four main quadrants
V = volume of counting area
Note: The total volume of the four quadrants is 0.0004mL. (Each quadrant is 0.0001mL.)
D = dilution factor. For this procedure the dilution factor is 2.

8.7. Calculate percent viability

8.7.1. Formula for percent viability: % viability = (live cell count/total cell count)*100

9. Attachments:

9.1. Figure 1: Diagram of hemacytometer and cover glass

9.2. Figure 2: Diagram of hemacytometer quadrants

Title: Trypan Blue Assay SOP

10. History:

Name	Date	Amendment
Kari Britt	26Jul05	Initial release
Deb Audino	04Apr08	College name change, format of history
Kari Britt	05Aug10	Proofreading, formatting and grammar edits throughout

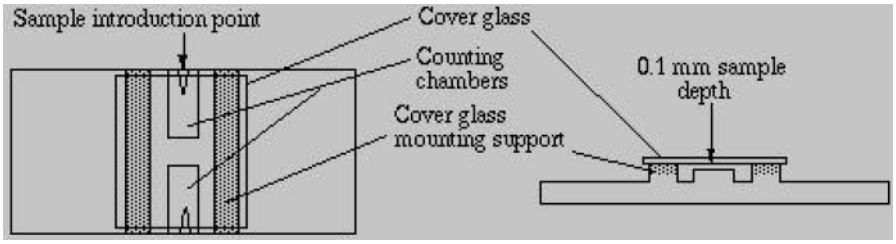


Figure 1: Diagram of hemacytometer and cover glass
Image: http://plaza.ufl.edu/johnaris/Protocols/MiscMethods/HemoCytometer.pdf

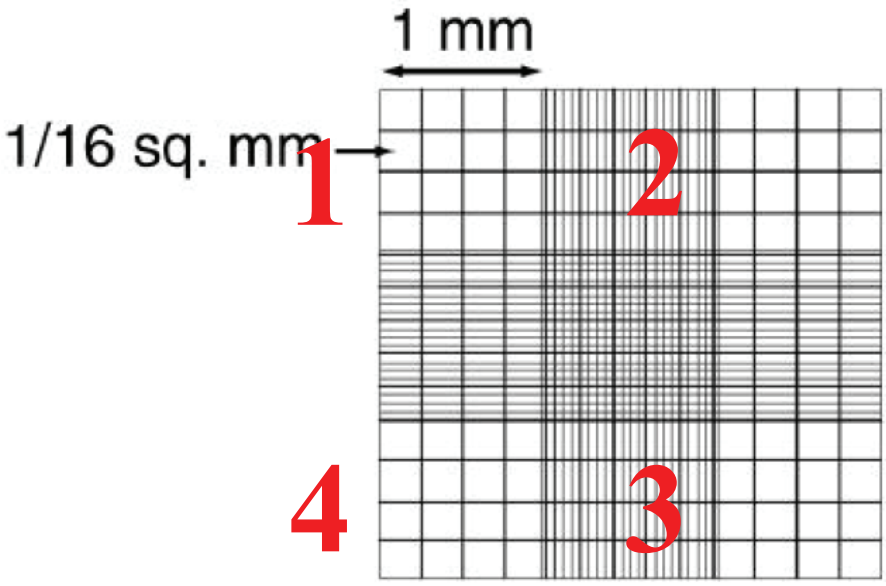


Figure 2: Diagram of hemacytometer quadrants

Great Bay Community College
320 Corporate Drive
Portsmouth, NH 03801

Kodak IBI Biolyzer Operation SOP

Approvals:
Preparer:_____ Deb Audino _____ Date _____03Apr08_____
Reviewer:_____Bob O"Brien_____ Date _____03Apr08_____

1. **Purpose:**
   1.1. Operation of the Kodak IBI Biolyzer Rapid Analysis.
2. **Scope:**
   2.1. Applies to the operation of the Kodak IBI Biolyzer Rapid Analysis System to track vital analyte consumption/production of living organisms.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that the SOP is performed as described and to update the procedure when necessary.
   3.2.It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Kodak IBI Biolyzer Manual.
   4.2. Kodak EKTACHEM DT pipette SOP.
5. **Definitions:**
   5.1. Analyte: chemical/substance being measured.
6. **Precautions:**
   6.1. Do not open spotting locator cover or the unit will have to reboot and warm-up again.
7. **Materials:**
   7.1. Vitros slides from the -20°C freezer.
   7.2. Kodak EKTACHEM DT pipette
   7.3. Kodak EKTACHEM DT pipette tips.
   7.4. charge cord for pipette.
8. **Procedure:**
   8.1. Instrument Preparation
      8.1.1.Turn on the instrument. Allow the instrument to warm up for 15-20 minutes prior to using.
      8.1.2.Plug in the charge cord to the Kodak pipettor. Make sure red plug shows in the window. If not, then depress the black-square button until it does.
      8.1.3.Remove the test slides from the freezer and/or refrigerator. Allow them to reach room temperature before using (15-30 minutes).
   8.2. Maintenance
      8.2.1.Perform daily maintenance prior to using the instrument by emptying the slide disposal box on the right rear of the unit and checking the paper level.
   8.3. Sample testing
      8.3.1.Enter the date in the Biolyzer by the following steps:
         8.3.1.1.Press the SHIFT then the SERVICE key.
         8.3.1.2.At the OPTION prompt, type "17" then ENTER.

Kodak IBI Biolyzer Operation SOP

8.3.1.3. Enter date as MM-DD-YY, then ENTER. NOTE: this will return you to the OPTION prompt.

8.3.1.4. Exit out of service mode by pressing the SHIFT then the SERVICE key.

8.3.2. Remove the slide to be tested from the foil package. NOTE: slides must be used within 15 minutes of opening a package.

8.3.3. Place the slide on the slide track, line up slide as shown on slide lever. Manually push the slide advance lever to move the slide into the spotting position. NOTE: console should read: "spot slide with fluid."

8.3.4. Enter ID of specimen (if necessary) by pressing SAMPLE ID key and enter sample name/number. If no ID is necessary the machine will automatically assign it a numerical number (ie. 1,2,3, etc.)

8.3.5. Using the Kodak EKTACHEM DT pipette, remove 10 µl of the test sample. Refer to the Kodak EKTACHEM DT pipette SOP for proper use.

8.3.6. Insert the pipette tip into the spotting locator (hole above slide) ensuring pipette is properly seated and dispense the sample. NOTE: The console should read: "Wait- slide being loaded."

8.3.7. Wait for the console to read: "Ready." Remove the pipettor from the locator and discard the pipette tip. Repeat steps 8.3.2 – 8.3.6 for each analyte to be tested. NOTE: slides will advance into slide disposal box.

8.4. Interpretation

8.4.1. The values will print shortly after procedure is completed

9. Attachments: N/A

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial release
Zach Bodah	18Feb05	Updated to 2005 SOP format
Deb Audino	04 Apr08	College name change

Great Bay Community College
320 Corporate Drive
Portsmouth, NH 03801

Kodak EKTACHEM DT Pipettor SOP

Approvals:
Preparer:_____ Deb Audino_____ Date _____03Apr08_____
Reviewer:_____Bob O'Brien_____ Date _____03Apr08_____

1. **Purpose:**
   1.1. Operation of the Kodak EKTACHEM DT Pipettor.
2. **Scope:**
   2.1. Applies to the Kodak EKTACHEM DT Pipettor used for accurately dispensing sample fluids into the Kodak IBI Biolyzer spotting locator.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that the SOP is performed as described and to update the procedure when necessary.
   3.2.It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. IBI Biolyzer Manual V. 3.0.
   4.2. Kodak IBI Biolyzer SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. Kodak EKTACHEM DT pipette tips.
   7.2. charge cord for pipette.
   7.3. Kodak IBI Biolyzer.
8. **Procedure:**
   8.1. Check Pipette
      8.1.1.Plug charge cord into the pipette and power ON.
      8.1.2.Check the pipette read window. The red indicator dot shows that the pipette is ready for operation. If the red dot is not showing, depress the button and release once.
   8.2. Insert Tip
      8.2.1.To attach a disposable tip, press the pipette firmly into one of the tips in the holder. The tip will click into place when it is seated.
   8.3. Aspirate fluid
      8.3.1.Hold pipette in vertical position.
      8.3.2.Insert tip into fluid, but not to the bottom of the fluid container.
      8.3.3.Depress button and release it (10µL of fluid is automatically drawn into pipette). Note: Do not depress the button more than once.
      8.3.4.A tone will be heard, which is the signal to withdraw the pipette. Remove the pipette from the fluid immediately.
      8.3.5.A second tone will be heard. This indicates that the pipette is stll drawing the fluid further into the tip, to prevent accidental loss of fluid.

Kodak EKTACHEM DT Pipettor SOP

8.3.6. Hold the pipette vertically whenever there is fluid in the tip. If you tilt the pipette or lay it on its side, fluid might enter the mechanism causing it to become clogged. If this occurs, clean the pipette immediately.

8.4. Remove excess fluid

8.4.1. To remove any droplets which may be clinging to the outside of the tip, take a laboratory tissue and wipe the outside of the tip in a light, quick motion. If the tip is not wiped, test results may be inaccurate. Visually check the fluid level.

8.5. Spot the slide with fluid

8.5.1. Depress and release the dispenser button. The Biolyzer console should read: "WAIT-slide being loaded." Wait for the console to read: READY before removing from the spotter locator.

8.6. Check pipette tip

8.6.1. Check that the fluid was completely dispensed from the tip.

8.6.2. Eject tip by pressing the eject button on the pipettor.

9. Attachments: N/A

10. History:

Name	Date	Amendment
Zach Bodah	18Feb05	Initial release
Deb Audino	04Apr08	College name change

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

Record Keeping Standards:

For each step in the batch record: the operator of the task will enter their initials (each operator has their own unique set of initials) and the date in the appropriate section(s) of the batch record. Another operator must initial and date in the appropriate section of the batch record to verify that the task was completed per SOP. No operator will verify their own work at any point. "If you didn't document it, you didn't do it!"

Batch records will be completed in blue or black ball point pen ONLY, and must be legible.

Any errors on a batch record will be crossed out with a single line through the error with the initials of the operator and the date. Corrections will be written in next to the crossed out error.

Use the following format to record dates: DDMMMYY. For July 10, 2006 use 10JUL06.

Use the 24 hour clock or "military time" to record time: 3:00pm would be written as 15:00.

Any and all deviations from a protocol or SOP, including abnormal results or retests performed, will be entered into the comments section at the end of each batch record. Be as detailed and specific as possible, include all steps taken before and/or after an abnormal reading, and provide an explanation for any deviations from a step.

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

1. Media Preparation		
Clean, assemble and autoclave two 100mL Bellco Spinner flasks per SOP. Spinner flask ID#_____Spinner flaskID#_____	Operator/Date	Verifier/Date
Obtain sterile Fetal Bovine Serum (FBS). Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Obtain sterile Ham's F12 Medium Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Sterilely add 90mL ± 1 mL of Ham's F12 Medium to a spinner flask. Repeat with the second spinner flask 100mL spinner flask ID#_____Vol of Ham's F12_____mL 100mL spinner flask ID#_____Vol of Ham's F12_____mL	Operator/Date	Verifier/Date
Sterilely add 10mL ± 1 mL of FBS to each spinner flask. 100mL spinner flask ID#_____Vol of FBS_____mL 100mL spinner flask ID#_____Vol of FBS_____mL	Operator/Date	Verifier/Date
Label spinner flasks as 90% Ham's F12, 10% FBS, [date], [group#], [operator initials].	Operator/Date	Verifier/Date
Place spinner flasks containing CHO cell media in the CO_2 incubator. Set the speed of the magnetic stirrer to the maximum setting that ensures an even vortexing of the culture without foaming.	Operator/Date	Verifier/Date
Verify that CO_2 is set to 5±0.5% and that temperature is set to 37±0.5ºC. CO_2_____% Temperature _____ ºC	Operator/Date	Verifier/Date
Check media for contamination after a minimum of 24 hrs. Elapsed Incubation Time _____ 100mL spinner flask ID_____ Contamination: Y / N (Circle) 100mL spinner flask ID_____ Contamination: Y / N (Circle)	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

2. Inoculation of Spinner Flasks		
Pre-warm the spinner Flasks containing CHO Cell Culture Medium at 37° C ± 0.5°C overnight.	Operator/Date	Verifier/Date
Remove two vials of CHO cells from storage in the -86°C freezer. Vial ID:_____ _____ Vial ID:_____ _____	Operator/Date	Verifier/Date
Sterilely transfer the entire contents of each 1mL vial of thawed CHO Cells into each of the previously prepared Spinner Flask containing 100mL CHO Cell Culture Medium using a 2mL sterile pipette. **Swirl** to mix.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number____________________

100mL Spinner Flask ID#________________________________

TIME (hours)	OD 650nm	pH	LIVE CELL Count	DEAD CELL Count	Viable cells/mL	Percent Viability	GLUCOSE (mg/dL)	LACTATE (mmol/L)
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number____________________

100mL Spinner Flask ID#________________________________

TIME (hours)	OD 650nm	pH	LIVE CELL Count	DEAD CELL Count	Viable cells/mL	Percent Viability	GLUCOSE (mg/dL)	LACTATE (mmol/L)
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

3. Solution and Buffer Preparation 500mL 1M ($NaHCO_3$) sodium bicarbonate 100mL of 1X PBS Phosphate buffered Saline		
Weigh out 21.0 ± 1 grams of ($NaHCO_3$) sodium bicarbonate. Label container: 1M $NaHCO_3$, [date], [initials], [group number], storage: room temp, disposal: drain. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve $NaHCO_3$ in 250 ± 5mL of deionized water using magnetic stirrer. Volume of water added _____ mL	Operator/Date	Verifier/Date
Dilute 10 ± 0.5mL of 10X stock solution, with 90 ± 5mL of deionized water in 100mL bottle using magnetic stirrer. Label container: 1X PBS, [date], [initials], [group number], storage: room temp, disposal: drain. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Volume of 10x PBS added:_____mL Volume of water added: _____mL	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

4. Assemble/Autoclave Bioreactor		
4.1. Assemble Vessel Stand		
Inspect the integrity of the large O-rings on the vessel stand and headplate. Replace if worn or cracked. Bioreactor ID #_____ Vessel stand O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Headplate O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
4.2. Assemble Headplate-Underside		
Inspect the integrity of the O-rings on the harvest tube, sparger, and the thermowell. Harvest tube O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Sparger O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Thermowell O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach harvest tube, sparger and thermowell. **Verify** that the sparger tube is aligned beneath the stirrer impeller.	Operator/Date	Verifier/Date
4.3. Attach Headplate to Vessel Stand.		
Place the headplate onto the vessel stand, positioning the holes on the outer edge of the headplate with the bolts on the vessel stand.	Operator/Date	Verifier/Date
Place the sample bottle assembly onto the bolt located by the 3 addition port and attach with a mill fastener.	Operator/Date	Verifier/Date
Secure the headplate with the 5 mill fasteners.	Operator/Date	Verifier/Date
4.4. Assemble Headplate – Topside		
Inspect the integrity of the O-ring in the condenser port of the headplate. Replace if worn or cracked. Condenser port O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced?: Yes / No (Circle one.)	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

Inspect the black seal at the bottom of the condenser underneath the retainer nut. Replace if worn or cracked. Condenser black seal worn or cracked? Yes / No (Circle one.) Black seal replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach condenser to headplate	Operator/Date	Verifier/Date
Remove protective cap from the bottom of the DO probe and inspect screen. Replace if damaged. Protective screen damaged? Yes / No (Circle one.) Protective screen replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Unscrew the membrane module from the bottom housing of the probe tip. Inspect the integrity of the O-ring. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Replenish DO electrolyte with O_2 electrolyte solution.	Operator/Date	Verifier/Date
Inspect the integrity of the O-ring at the top of the stainless steel DO probe. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Inspect the black seal at the top of the DO probe under the retainer nut. Replace if worn or cracked. Black seal worn or cracked? Yes / No (Circle one.) Black seal replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach DO probe to the headplate.	Operator/Date	Verifier/Date
Calibrate the pH probe. pH 7 Buffer Manufacturer:_____ Catalog number: _____ Lot number: _____ Expiration date:_____ pH 4 Buffer Manufacturer:_____ Catalog number: _____ Lot number: _____ Expiration date:_____	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

Record pH calibration values. pH 7.00 standard: pH value _____ temp_____ pH 4.00 standard: pH value _____ temp_____ Slope from the Display _____Expected value: 0.95-1.05 Offset from the Display_____Expected value: < ±0.3	Operator/Date	Verifier/Date
Inspect the integrity of the O-ring at the top of the pH probe. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Inspect the black seal at the top of the pH probe under the retainer nut. Replace if worn or cracked. Black seal worn or cracked? Yes / No (Circle one.) Black seal replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach pH probe to the headplate.	Operator/Date	Verifier/Date
4.5. Attach Filters and Tubing		
Place silicone tubing on the Sparger tube,Condenser top outlet, and CO_2 overlay port. **Use** a small piece of silicon tubing to connect together 2 of the ports on the 3 port addition. **Connect** the pharmed tubing from the feed bottle to the 3 addition port. **Connect** the sample bottle tubing to the harvest tube.	Operator/Date	Verifier/Date
Clamp off all tubing (near the headplate) except the condenser top outlet. The condenser top outlet must remain unclamped to release pressure during autoclaving.	Operator/Date	Verifier/Date
Close all open ends with glass wool and cover with aluminum foil (including the harvest tube and sample bottle assembly tubing).	Operator/Date	Verifier/Date
Autoclave per SOP. Autoclave at 121°C for 20 minutes, using slow exhaust.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

5. Media Preparation and Addition / Run Preparation		
Place the bioreactor and a sterile funnel in the Biological Safety Cabinet and expose to UV light for 20-30 minutes.	Operator/Date	Verifier/Date
Add approximately 6mL of 200mM Glutamine and 10mL of 10mg/mL gentamycin to a 1L bottle of ProCHO4 media. Pour into bioreactor. ProCHO4 media: Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Glutamine: Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount added:_____mL Gentamicin: Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount added:_____mL	Operator/Date	Verifier/Date
Verify that glycerol has been added to the thermowell with the Pt-100 temperature probe. Add more if necessary.	Operator/Date	Verifier/Date
Verify that thermal blanket is wrapped around the vessel and plugged into the ADI 1025 unit.	Operator/Date	Verifier/Date
Input the following limits per the process SOP and activate the control loops. **Parameter** **Upper limit** **Lower limit** pH 7.3 7.1 Temperature 38 36 DO 52 48 Temperature 38 36 Agitation 76 74	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

Calibrate DO probe per Applikon Bioreactor Operation SOP. **Note:** Allow DO probe to polarize for at least 6 hours before performing calibration. **Record** slope: _____ Expected values are: 8-15 at 37°C or 10-20 at 25°C	Operator/Date	Verifier/Date
Turn on Air supply at regulator Tank pressure _____ Tank Volume_____	Operator/Date	Verifier/Date
Turn on CO_2 supply at regulator to the bioreactor. Tank pressure _____ Tank Volume_____	Operator/Date	Verifier/Date
Check the media for contamination before inoculation. Contamination? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Inoculate bioreactor when the 100mL suspension culture of CHO cells reaches a concentration of about 1,000,000 cells/mL. Volume of culture added:_____	Operator/Date	Verifier/Date
Turn on computer and open BioXpert Lite software per Applikon Bioreactor Operation SOP. **Name** the file. File Name: _____	Operator/Date	Verifier/Date
Ensure the computer is communicating with the controller per the Applikon Bioreactor Operation SOP. **Click** the OK button to begin the data collection process. **IMPORTANT** – In the On-Line Session window **DO NOT CLICK ON *END***. This will end the on-line session and stop collecting data.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number____________________

Applikon Bioreactor ID#____

TIME (hours)	OD 650nm	pH	LIVE CELL Count	DEAD CELL Count	Viable cells/mL	Percent Viability	GLUCOSE (mg/dL)	LACTATE (mmol/L)
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

6. Ending a Run		
Save the file for the run. File Name: _____	Operator/Date	Verifier/Date
Turn off each control loop. Turn off the supply of Air the ADI1025 controller. Turn off the supply of CO_2 supplied to the ADI1025 controller.	Operator/Date	Verifier/Date
Aseptically remove the culture through the harvest port.	Operator/Date	Verifier/Date
Clean the pH , DO, and the Pt-100 probes with a 10% bleach solution, and rinse with DI water. Place protective caps on the pH probe. Place protective caps on the DO probes.	Operator/Date	Verifier/Date
Clean the bioreactor.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
7. Harvest and Preparation of Working Cell Bank		
Using a 25mL sterile pipet, divide the 500mL suspension culture into about 20 sterile 30mL centrifuge tubes. (about 25mL per tube).	Operator/Date	Verifier/Date
Centrifuge tubes for 10min at 2000rpm. (If using the Sigma 2K15 choose program 75). BE SURE TO BALANCE TUBES WHEN LOADING ROTOR.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

8. Prepare storage menstrum:		
In a container capable of holding >50mL add 40mL ± 1mL of Ham's F12 manufacturer: _____ lot number: _____ expiration date: _____ volume Ham's F12:_____	Operator/Date	Verifier/Date
Into the same container add 5mL ± 0.5mL of FBS manufacturer: _____ lot number: _____ expiration date: _____ volume FBS:_____	Operator/Date	Verifier/Date
Into the same container add 5mL ± 0.5mL of glycerol manufacturer: _____ lot number: _____ expiration date: _____ volume FBS:_____	Operator/Date	Verifier/Date
Filter sterilize and label bottle as CHO storage Menstrum with the date.	Operator/Date	Verifier/Date
Following centrifugation, decant tPA containing medium into sterile 250mL bottles. **Label** bottles as unpurified tPA in Ham's F12/FBS and date. **Store** supernatant in the refrigerator at 2-8°C.	Operator/Date	Verifier/Date
Add about 1mL of storage menstrum to each centrifuge tube to resuspend the pelleted CHO cells. **Sterilely dispense** 1mL ± 0.1mL aliquots into sterile 1.5mL cryovials. **Label** in the following manner using a cryopen: CHO (ATCC CRL-9606), [DATE], [INITIALS]. **Place** in a styrofoam tube rack, label container same as cryovials. **Store** at -85°C.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

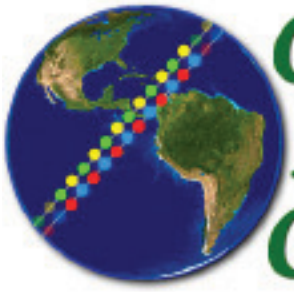
9. Prepare Growth Curves		
Plot OD, pH, viable cells, glucose, and lactatevs. time (use 2 y-axes). Attach graph to Batch Record.	Operator/Date	Verifier/Date
Determine growth rate and doubling time of the 50mL and 500mL cultures (Show calculation) Growth Rate 100mL ID#_____is_____ Growth Rate 100mL ID#_____is _____ Growth Rate bioreactor ID#_____is _____	Operator/Date	Verifier/Date
Send samples to QC Chemistry department for ELISA and Activity Assays.	Operator/Date	Verifier/Date
Attach QC data to the batch record.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Manufacturing Technician (Upstream)

REF	Key Functions & Tasks (Upstream Manufacturing Technician)
1	**Work in compliance with EH&S.**
1.a	Wear appropriate personal protective equipment.
1.b	Work in controlled environments.
1.c	Participate in emergency drills and emergency response teams.
1.d	Identify unsafe conditions and take corrective action.
1.e	Appropriately and safely access production equipment.
1.f	Handle, label, and dispose of hazardous / biohazard materials.
1.g	Access and utilize MSDS.
1.h	Perform permitting procedures.
1.i	Carries out operations with attention to OSHA and EPA regulations, and other applicable state and federal regulations.
1.j	Keeps work areas clean and safety equipment in order.
1.k	Participate in all company safety training and audits as required.
1.l	Assists with waste treatment operations.
2	**Work in compliance with cGMPs.**
2.a	Assists in environmental monitoring activities.
2.b	Follow SOPs for all operations.
2.c	Records process data and completes batch records as required.
2.d	Maintain equipment logbooks.
2.e	Control and receipt of raw materials.
2.f	Maintain training documentation.
2.g	Maintain equipment and processes in a validated state.
2.h	Working in controlled/classified areas (gowning, aseptic technique).
2.i	Ensure appropriate flow of personnel, equipment, and materials.
2.j	Change control for process, equipment, and documentation.
2.k	Label and apply status to equipment and materials.
2.l	Identify and report exception events and CAPA.
3	**Clean and maintain production areas.**
3.a	Housekeeping / pest control.
3.b	Sanitize and clean of controlled spaces.
3.c	Preparation of cleaning materials and solutions.
3.d	Assist in environmental monitoring for routine and changeover operations.
3.e	Document cleaning.
4	**Maintain effective communication.**
4.a	Deliver shift change update.
4.b	Communicate with coworkers and/or customers to ensure production or service meets requirements.
4.c	Suggest continuous improvements.
4.d	Coordinate with work teams / internal customers.
4.e	Maintain security and confidentiality.
4.f	Respond appropriately to internal auditors and external inspectors.
4.g	Assist in writing, reviewing, and commenting on technical documents.

REF	Key Functions & Tasks (Upstream Manufacturing Technician)
5	**Prepare process materials.**
5.a	Weigh, dispense, and label raw materials for use in production.
5.b	Dispense consumables and intermediates.
5.c	Control and reconcile inventory with enterprise control system (MRP, SAP, manual database).
5.d	Prepare and sterilize buffers and solutions.
5.e	Sample and test buffers and solutions.
5.f	Transfer buffers and solutions to use point.
5.g	Prepare filters for use.
5.h	Prepare, pasteurize / sterilize, and titrate media and feed solutions.
5.i	Manage chromatography resins.
6	**Prepare equipment.**
6.a	Clean CIP vessels, transfer lines, and filter trains .
6.b	Clean COP equipment (or sonicator).
6.c	Depyrogenate components and equipment.
6.d	Sterilize SIP vessels, transfer lines, and sampling ports.
6.e	Perform pressure test.
6.f	Prepare and assemble components and equipment.
6.g	Autoclave components and equipment.
6.h	Perform WFI flush of transfer lines.
6.i	Perform scheduled sanitizations of hoods.
6.j	Prepare and standardize probes and ancillary instruments.
6.k	Prepare, assembly, and integrity test filters.
6.l	Visually inspect equipment.
6.m	Maintain equipment logs and status tags.
6.n	Complete, review and approve equipment process records.
7	**Perform basic manufacturing operations.**
7.a	Perform processes following batch records, validation protocols, and/or SOPs.
7.b	Maintains and controls processes in an automated control environment.
7.c	Record process data.
7.d	Inspect materials at all stages of process to determine quality or condition.
7.e	Participate in the installation, modification, and upgrade of equipment.
7.f	Operate, monitor, and maintain equipment, tools, and workstation.
7.g	Recognize and respond appropriately to atypical events.
7.h	Participate in troubleshooting and root cause analysis of operations.
8	**Perform upstream manufacturing operations.**
8.a	Work in an aseptic environment (laminar flow hood / biosafety cabinet / cleanrooms).
8.b	Perform vial thaw from a working cell bank.
8.c	Perform cell culture expansion.
8.d	Monitor cell concentration by cell counting or measuring OD.
8.e	Inoculate seed reactor.
8.f	Transfer of seed culture to production reactors.
8.g	Monitor and control growth of cells in batch, fed-batch, and perfusion reactors.
8.h	Perform aseptic additions of media, solutions, and/or gases to reactors.
8.i	Perform CIP/SIP of bioreactors.
9	**Perform Sampling.**
9.a	Prepare sample port for aseptic sampling.
9.b	Obtain in-process samples according to batch records or sampling plans.
9.c	Label samples appropriately.
9.d	Record sample collection and distribution (storage and chain of custody).
9.e	Perform in-process chemical and/or microbiological tests.

Upstream - HSA



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Title: Process Controlled Fed-Batch Fermentation of Recombinant HSA Secreting *Pichia pastoris* SOP

Approvals:

Preparer:_____Kari Britt_____ Date ____01Apr09_____
Reviewer:_____Sonia Wallman_____ Date____ 01Apr09_____

1. **Purpose:**
   1.1. To produce a fed batch culture of yeast cells.
2. **Scope:**
   2.1. Applies to producing a process controlled fed batch culture of *Pichia pastoris* recombinant for human serum albumin.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. pH Meter SOP
   4.2. autoclave SOP
   4.3. shaking incubator SOP
   4.4. spectrophotometer SOP
   4.5. microscope SOP
   4.6. Gram stain SOP
   4.7. Biolyzer SOP
   4.8. BioFlo 3000 SOP
   4.9. HSA ELISA SOP
   4.10. centrifuge SOP
   4.11. Cino, Julia, *High Yield Protein Production from Pichia pastoris Yeast: A Protocol for Benchtop Fermentation.* May 1999 American Biotechnology Laboratory.
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Use BL2 safety measures and discard culture waste in biohazard containers.
   6.2. Ammonium hydroxide is extremely corrosive. Wear safety glasses and transfer into containers in a fume hood. It is extremely damaging to eyes and mucous membranes. It causes burns. Avoid contact with skin. Harmful if swallowed or inhaled.
7. **Materials:**
   7.1. *Pichia pastoris* expressing Human Serum Albumin (Yeast strain: GS 1 15/HIS+/MUT-/SEC HSA by Invitrogen is recommended.)
   7.2. BioFlo 3000 bench-top fermenter (New Brunswick Scientific Co., Inc.), 5 liter working volume
   7.3. visible microscope with 1000x magnification
   7.4. shaking incubator (37^{o}C and 30^{o}C)
   7.5. autoclave

Title: Process Controlled Fed-Batch Fermentation of Recombinant HSA Secreting *Pichia pastoris* SOP

7.6. water bath (30°C)
7.7. pH meter
7.8. spectrophotometer
7.9. centrifuge
7.10. Biolyzer or glucose test strips (Such as: Urine Reagent Strips from LW Scientific, Item Number: URS-01PR-GL77)
7.11. Antifoam A (optional)
7.12. potassium phosphate dibasic (K_2HPO_4)
7.13. potassium phosphate monobasic (KH_2PO_4)
7.14. glucose
7.15. yeast nitrogen base (YNB) without amino acids
7.16. yeast extract
7.17. peptone
7.18. five 500mL shake flasks
7.19. 100mL glass bottle
7.20. 1L flask
7.21. 100% methanol feed solution (1 Liter)
7.22. 30% ammonium hydroxide solution (500mL)
7.23. compressed air
7.24. Gram stain kit

8. Procedure:

8.1. **Media Preparation for Seed Flask Cultures**

8.1.1. Prepare 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone.

8.1.1.1. Dissolve 1.3 ± 0.05g potassium phosphate dibasic (K_2HPO_4) and 5.8 ± 0.05g potassium phosphate monobasic (KH_2PO_4) in 500mL±5mL deionized water in a 1L vessel to make 0.1M potassium phosphate buffer.

8.1.1.2. Adjust 0.1M potassium phosphate buffer to pH 6 ±0.1.

8.1.1.3. Add 5±0.5g yeast extract, 10±0.5g peptone, and 10g±0.5g glucose to the 0.1M potassium phosphate buffer and stir to dissolve.

8.1.1.4. Transfer 90mL of the 0.1M Potassium Phosphate Media with 1% Yeast Extract and 2% Peptone into five 500mL shake flasks so that each flask contains 90mL media.

8.1.1.5. Transfer 36mL of the media into a 100mL autoclavable bottle.

8.1.1.6. Autoclave the 500mL shake flasks and 100mL bottle containing media per autoclave SOP.

8.1.1.7. Prepare 100mL 10X Yeast Nitrogen Base (YNB) Solution without amino acids.

8.1.1.7.1. Weigh out 6.7±0.02g YNB without amino acids and combine with 100±1mL deionized water in a 500mL vessel.

8.1.1.7.2. Filter sterilize the 10X YNB and label as: Sterile Filtered 10X YNB, [date], [initials], Store: 2-8°C, Dispose: drain.

Title: Process Controlled Fed-Batch Fermentation of Recombinant HSA Secreting *Pichia pastoris* SOP

8.1.1.8. Aseptically add 10mL 10X YNB to each of the five autoclaved and COOLED shake flasks of media containing 90mL of 0.1M Potassium Phosphate Media with 1% Yeast Extract and 2% Peptone.

8.1.1.9. Aseptically add 4mL YNB to the 100mL bottle containing 36mL 0.1M Potassium Phosphate Media with 1% Yeast Extract and 2% Peptone and store at 4°C until needed to blank the spectrophotometer.

8.1.1.10. Label the five shake flasks as: 0.1M Potassium Phosphate Media, pH 6, 1X YNB, with 1% Yeast Extract and 2% Peptone, [date], [group], [initials], Store: 2-8°C, Dispose: drain.

8.1.1.11. Label the 100mL bottle as: 0.1M Potassium Phosphate Media, pH 6, 1X YNB, with 1% Yeast Extract and 2% Peptone, [date], [initials], Blanking Media for Spectrophotometer, Store: 2-8°C, Dispose: drain.

8.1.1.12. Proof the media in the shake flasks at 37°C ± 0.5C and shaking at approximately 200rpm for a minimum of 24 hours.

8.1.1.13. Visually check the media in the shake flasks for contamination. If no contamination is present, four of them can be used for inoculation and one of them should be stored at 2-8°C until the media is needed for cryopreservation. Add to the label: For Cryopreservation of *Pichia pastoris*.

8.1.1.14. If the media in any of the shake flasks becomes contaminated, add bleach and dispose down the drain.

8.2. **Seed Flask Culture**

8.2.1. Thaw the contents of four 1mL cryovials (one vial per shake flask) of *Pichia pastoris* cells in 30°C water bath. Record the Vial ID including the passage number of the cells. Passage number is indicated as P[#].

8.2.2. Prepare the biological safety cabinet (BSC) per the BSC SOP.

8.2.3. Spray the outside of the cryovials and the autoclaved 500mL shake flasks containing 100mL 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone with 70% isopropanol, allow to dry for at least 30 seconds, and place them in the BSC.

8.2.4. Spray all items that will be needed for step 8.2.5 with 70% isopropanol and allow to dry for at least 30 seconds before placing in the BSC.

8.2.5. Sterilely transfer the contents of each vial to an autoclaved shake flask containing media in the BSC.

8.2.6. Remove the shake flasks from the BSC and label as: Pichia Inoculum [group], [date], [initials], Dispose: Autoclave then drain.

8.2.7. Incubate shake flasks for 24-48 hours at 30°C and shaking at approximately 200 rpm. Note: Shake flask caps should be loose while shaking to promote aeration of the culture.

8.3. **Sampling the Seed Flask Culture**

Reminder: Record all sampling results in the batch record and in the data table at the end of the batch record as needed.

Title: Process Controlled Fed-Batch Fermentation of Recombinant HSA Secreting *Pichia pastoris* SOP

8.3.1. Aseptically remove a 2mL sample from each seed flask and place into a corresponding labeled cuvette.
Note: Do not label cuvettes in an area that would interfere with OD reading.

8.3.2. Take an OD reading of cultures at 600nm per the spectrophotometer SOP using the Blanking Media as the blank. OD absorbance should be between 2 and 6.
Reminder: If the initial sample OD reading is greater than 1.0, the sample should be diluted until it reads below 1.0 and then multiply by the dilution factor to obtain the absorbance value.

8.3.3. Prepare a Gram stain of each culture per the Gram Stain SOP and examine the cultures for contamination using a microscope per microscope SOP.

8.3.4. Transfer the sample to a test tube to measure the pH per the pH meter SOP.

8.3.5. Transfer 1.5mL of sample to a microfuge tube and centrifuge at high speed for 5 minutes.

8.3.6. Remove the supernatant and transfer it to a clean microfuge tube.

8.3.7. Label the tube as: Seed Flask Sample, HSA, [lot number], [date], [group], [initials] and store at 2-8°C until needed for SDS-PAGE and ELISA.

8.4. **Media Preparation for Bioreactor**

8.4.1. Prepare 2.7 liters of 0.1M Potassium Phosphate Media, pH 6 and 300mL 10X YNB for use in the bioreactor.
Note: Yeast extract and peptone are purposefully left out of the bioreactor media to help reduce foaming. However, if you experience poor cell growth, 1% yeast extract and 2% peptone can be added to the bioreactor media in future runs. If you choose to use yeast extract and peptone, then be prepared to use an antifoaming agent such as Antifoam A in case of excess foaming during the run.

8.4.1.1. Dissolve 2.3±0.05g potassium phosphate dibasic and 10.4±0.05g potassium phosphate monobasic in 900±10mL deionized water in a 2L flask to make 0.1M potassium phosphate buffer, pH 6.

8.4.1.2. Adjust 0.1M potassium phosphate buffer to pH 6 ±0.1.

8.4.1.3. Add 20±0.5g glucose to the 0.1M potassium phosphate buffer and stir to dissolve.

8.4.1.4. Label flask as: 0.1M Potassium Phosphate Media, pH 6, [date], [initials], Store: 2-8°C, Dispose: drain.

8.4.1.5. Repeat steps 8.4.1.1 though 8.4.1.4 two times to make 2.7L of 0.1M Potassium Phosphate Media, pH 6.

8.4.1.6. Prepare 300mL 10x Yeast Nitrogen Base (YNB) Solution without amino acids.

8.4.1.6.1. Weigh out 20.1±0.05g YNB without amino acids and combine with 300±5mL deionized water in a 500mL vessel.

8.4.1.6.2. Filter sterilize the 10X YNB and label as: Sterile Filtered 10X YNB, [date], [initials], Store: 2-8°C, Dispose: drain.

8.5. **Assemble BioFlo 3000 per BioFlo 3000 SOP.**

8.5.1. Clean all bioreactor parts per BioFlo 3000 SOP.

Title: Process Controlled Fed-Batch Fermentation of Recombinant HSA Secreting *Pichia pastoris* SOP

8.5.2. Assemble the vessel per BioFlo 3000 SOP.
8.5.3. Assemble the headplate (underside) per BioFlo 3000 SOP.
8.5.4. Aseptically add 2.7L of 0.1M Potassium Phosphate Media, pH 6 to the vessel per BioFlo 3000 SOP.
8.5.5. Attach the headplate to the vessel per BioFlo 3000 SOP.
8.5.6. Assemble the headplate (top side) per BioFlo 3000 SOP.
8.5.7. Connect the bioreactor to the cabinet per BioFlo 3000 SOP.
8.5.8. Calibrate the pH probe per the BioFlo 3000 SOP.
8.5.9. Install Dissolved Oxygen probe per BioFlo 3000 SOP.
8.5.10. Attach tubing per BioFlo 3000 SOP.
8.5.11. Autoclave the entire assembly for at a minimum of 121°C and at least 30 minutes per BioFlo 3000 SOP and autoclave SOP.
8.5.12. Once the bioreactor vessel has cooled, aseptically add 300mL of filtered 10X YNB through the inoculation port of the headplate (See section 9 of the BioFlo 3000 SOP for the position of the inoculation port in the headplate.).

8.6. **Prepare Feed Solutions for BioFlo 3000**
8.6.1. Assemble two 1L flasks (each with a sidearm) for feed solutions.
8.6.1.1. Insert a 2mL glass pipet into a rubber stopper. Apply a small amount of deionized water to the outside of the pipet before inserting, if needed. Repeat this step with a second pipet and rubber stopper.
8.6.1.2. Insert the rubber stopper with glass pipet into the top of a 1L flask with a sidearm. Repeat this step with the second rubber stopper and 1L flask.
8.6.1.3. Adjust the height of the glass pipets so that the tips are just above the bottom of the flasks.
8.6.1.4. Attach tubing with an air filter to the side arm of each flask.
8.6.2. Autoclave the two assembled feed solution flasks per autoclave SOP.
8.6.3. Allow the feed solution flasks to cool to room temperature before adding feed solutions.
8.6.4. Aseptically pour approximately 500mL of 30% ammonium hydroxide (NH_4OH) into an assembled feed solution flask. CAUTION: Wear safety glasses and pour in a fume hood.
8.6.5. Aseptically pour approximately 1L of 100% methanol into an assembled feed solution flask.

8.7. **Prepare the bioreactor for operation per the BioFlo 3000 SOP.**
8.7.1. When prompted by the BioFlo 3000 SOP, input the working temperature into the control panel of the bioreactor.
8.7.1.1. Desired Working Temperature: 30°C
8.7.2. Set up the feed solution flask containing 30% ammonium hydroxide (NH_4OH) solution on Feed 1 per BioFlo 3000 SOP. Ammonium hydroxide is a basic solution.
8.7.3. Set up the feed solution flask containing 100% methanol solution on Feed 2 per BioFlo 3000 SOP.

Title: Process Controlled Fed-Batch Fermentation of Recombinant HSA Secreting *Pichia pastoris* SOP

8.7.4. When prompted by the BioFlo 3000 SOP, input the desired pH into the control panel of the bioreactor.
8.7.4.1. Desired pH: 6.0
8.7.5. Calibrate the dissolved oxygen probe per BioFlo 3000 SOP.
8.7.6. Set DO mode to Controlling by Agitation Only per BioFlo 3000 SOP.
8.7.6.1. Set minimum agitation rpm to 200.
8.7.6.2. Set maximum agitation rpm to 1000.
8.7.6.3. Set agitation to DO control mode.
8.7.6.4. Set the dissolved oxygen level (DO) to 30%.
8.7.7. Disregard the use of the foam sensor.

8.8. **Fermentation Procedure**

8.8.1. Set up and start the BioCommand Lite program according to the instructions in the Fermentation Procedure section of the BioFlo 3000 SOP.

8.9. **Bioreactor inoculation**

Note: If excess foaming occurs during the run, an antifoaming agent can be added aseptically through the addition port. Dilute the antifoaming agent per the manufacturer's instructions. Alternatively, 1mL of soybean oil can be used as an antifoaming agent. There may be a small risk of contamination if you choose to use soybean oil. Therefore, aseptically remove the oil from a brand new container.

8.9.1. Allow all of the bioreactor time to reach all of its setpoints.
8.9.2. Choose the seed flask culture that has the highest OD and has NO contamination to inoculate the BioFlo 3000. Aseptically add the contents of the chosen flask through the inoculation port. The contents of more than one seed flask (with NO contamination) can be added if the OD readings are below 4.
Note: Unused seed flask cultures can be used for cryopreservation as directed in step 8.13.
8.9.3. Immediately take a sample of the culture following the instructions below.

8.10. **Sampling the Bioreactor Culture**

Reminder: Record all sampling results in the batch record and in the data table at the end of the batch record as needed.

8.10.1. Sample the culture a minimum of once per day.
8.10.1.1. Attach bulb to the sample port of the BioFlo 3000 (Be sure there is glass wool in tube before attaching.) and remove 2-8mL of culture.
8.10.1.2. Take an OD reading at 600nm per the spectrophotometer SOP using water as a blank for the spectrophotometer. Record the OD reading on the data table. **Reminder:** If the initial sample OD reading is greater than 1.5, the sample should be diluted until it reads below 1.5 and then multiply by the dilution factor to obtain the absorbance value.
8.10.1.3. Measure the glucose level per the Biolyzer SOP. Record the glucose reading on the data table.
8.10.1.4. Transfer 1.5mL of sample to a microfuge tube and centrifuge at high speed for 5 minutes.

Title: Process Controlled Fed-Batch Fermentation of Recombinant HSA Secreting *Pichia pastoris* SOP

8.10.1.5. Remove the supernatant and transfer to a clean microfuge tube.
8.10.1.6. Label the tube as: Bioreactor Sample, HSA, [lot number], [date], [group], [initials] and store at 2-8°C until needed.
8.10.1.7. After 24-72 hours and when glucose levels reach an undetectable level move to Stage 2 of growth (described below). Ideally, the OD absorbance value should be approaching or greater than 20, but the culture can be can be moved into Stage 2 even if the OD is lower than 20.
8.10.1.8. From this point on, glucose levels do not need to be measured.

8.11. **Bioreactor Growth Stages**

8.11.1. **Stage 1: Batch Growth**

8.11.1.1. Maintain starting conditions for approximately 24-72 hours.
8.11.1.2. When the OD reaches approximately 20 and glucose levels are undetectable, move to Stage 2.

8.11.2. **Stage 2: Fed-Batch Production of Human Serum Albumin**

8.11.2.1. Change the setpoint for Feed 2 (100% methanol) to 1 (for 1%) by following the directions to "Activate additional feed loops at the appropriate time as indicated by the process SOP" section in the BioFlo 3000 SOP.
8.11.2.2. Feed for 12-48 hours and then harvest the culture.

8.12. **Data Collection and Cell Harvest**

8.12.1. Retrieve data generated by Biocommand Lite per BioFlo 3000 SOP.
8.12.2. Using the sampling assembly, collect 1L of culture into sterile bottles through the harvest port.
8.12.3. Transfer approximately 50mL of the culture into individual centrifuge tubes.
8.12.4. Centrifuge at approximately 3000xg for 5-8 minutes.
8.12.5. Remove the supernatant by pouring into sterile bottles.
8.12.6. Store supernatant at 2-8°C for use in downstream processing SOPs.
8.12.7. Harvest remaining culture through the harvest port into bottles for autoclaving, then disposal.
8.12.8. Shut down and clean the BioFlo 3000 per BioFlo 3000 SOP.

8.13. **Cryopreservation**

Note: It is recommended to cryopreserve cells from the unused seed flask cultures rather than the bioreactor, since treatment with methanol can be toxic to the cells.

8.13.1. Autoclave 50mL of 100% glycerol in a 100mL bottle per autoclave SOP.
8.13.2. Prepare the Biological Safety Cabinet (BSC) per the BSC SOP.
8.13.3. Spray the outside of all items that will be needed for steps 8.13.4 through 8.13.10 with 70% isopropanol, allow to dry for at least 30 seconds, and then place in the BSC.
8.13.4. In the BSC, sterilely transfer about 50mL of the culture into individual centrifuge tubes.
8.13.5. Remove the centrifuge tubes from the BSC to centrifuge at approximately 3000xg for 5 minutes.

Title: Process Controlled Fed-Batch Fermentation of Recombinant HSA Secreting *Pichia pastoris* SOP

8.13.6. Spray the outside of the tubes with 70% isopropanol and allow to dry for at least 30 seconds before returning them to the BSC.
8.13.7. In the BSC pour off the supernatant into a waste container.
8.13.8. Sterilely add 11mL of autoclaved glycerol to the 100mL of 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone set aside for cryopreservation in step 8.1.1.13. to make the storage media.
8.13.9. Aseptically add 5mL of the storage media to each centrifuge tube and resuspend the pelleted *Pichia* cells.
8.13.10. Aseptically dispense 1mL aliquots to sterile 1.5mL cryovials. Label the cryovials: P. pastoris, HSA, [date], [initials], P[#]. Increase the passage number by one from the recorded Vial ID used in the seed flask culture.
8.13.11. Place cryovials in a Styrofoam tube rack. Label container: P. pastoris, HSA, Working Cell Bank, [date], [initials], P[#]. Store at -86°C.

9. Attachments:

9.1. Data table

10. History:

Name	Date	Amendment
Deb Audino Laura Hyson	31Aug07	Initial Release
Deb Audino	04Apr08	College name change and rearranged steps.
Kari Britt	01Apr09	Added in descriptions of growth stages and cryopreservation directions. Changed media components for the seed flask culture. Added additional steps to the sampling sections. Added information regarding antifoam. Rearranged steps for consistency to Batch Record and other upstream processing documents. Also made general grammar and formatting edits as needed throughout the document.

Title: Process Controlled Fed-Batch Fermentation of Recombinant HSA Secreting *Pichia pastoris* SOP

Elapsed Time (Hours)	pH	Temp (^{o}C)	%DO2	Agitation (rpm)	Methanol Feed	OD (600nm)	Glucose (mg/dL)	Operator/Verifier

Title: New Brunswick BioFlo 3000 Bioreactor SOP

Approvals:
Preparer: ___Kari Britt_____ Date _____15May09_____
Reviewer: __Bob O'Brien_____ Date _____15May09_____

1. **Purpose:** Operation of the New Brunswick BioFlo 3000 Bioreactor.
2. **Scope:** Applies to growing yeast or bacteria in the New Brunswick BioFlo 3000 Bioreactor.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. New Brunswick BioFlo 3000 Bioreactor instrumentation manual
   4.2. BioCommand Lite Software Manual
   4.3. autoclave SOP
5. **Definitions: N/A**
6. **Precautions: N/A**
7. **Materials:**
   7.1. pH 4.0 buffer standard
   7.2. pH 7.0 buffer standard
   7.3. laboratory tissues
   7.4. silicone tubing
   7.5. PharMed tubing
   7.6. clamps
   7.7. aluminum foil
   7.8. air filters
   7.9. glass wool
   7.10. probe stand with clamps
   7.11. glycerol
   7.12. laboratory glassware detergent
   7.13. deionized water
   7.14. DO electrolyte
   7.15. 3M potassium chloride (KCl) solution for pH probe storage
   7.16. bleach
   7.17. Optional: antifoaming agent (such as Antifoam A)
   7.18. personal computer to run BioCommand Lite Software and Microsoft Excel
   7.19. **Bioreactor**
      7.19.1. pH probe
      7.19.2. DO probe
      7.19.3. RTD temperature probe
      7.19.4. harvest tube
      7.19.5. sparger tube
      7.19.6. thermowell tube

Title: New Brunswick BioFlo 3000 Bioreactor SOP

7.19.7. glass vessel
7.19.8. condenser
7.19.9. feed solution flask assembly
7.19.10. sampler assembly
7.19.11. heat exchanger base
7.19.12. baffle
7.19.13. thumb screws

8. Procedure:

Note: Refer to Section 9 (Attachments) for diagrams of the bioreactor parts as needed throughout this procedure.

8.1. **Clean All Bioreactor Parts**

8.1.1. Gather all bioreactor vessel parts, including the DO probe, pH probe and calibrating buffers.

8.1.2. Clean all bioreactor vessel parts (except pH and DO probes) with a soft cloth, warm water, and glassware detergent. Rinse thoroughly with warm tap water, and then with several changes of deionized water.
Note: A soft brush may be used on difficult to clean parts of the bioreactor vessel.

8.1.3. Gently clean the pH and DO probes using a soft cloth and deionized water. Be careful not to harm the sensory tips of these probes.

8.1.4. Rinse the inside of the sparger tube to remove clogs and prevent damage to the bioreactor.

8.2. **Assemble the Vessel**

Note: All fittings are hand-tightened except the DO probe adapter.

8.2.1. Attach the DO, pH, and temperature cables to their appropriate outlets on the front side of the cabinet of the BioFlo 3000.

8.2.2. If necessary, insert the plastic plugs into the holes in the glass vessel.

8.2.2.1. Make sure that the top steel ring is lifted above the holes before inserting the plugs or the headplate will not be able to be attached.

8.2.2.2. Insert the plastic bolt through the hole from the inside of the glass vessel. Place the rubber plug over the end of the bolt on the outside of the vessel (with the tapered side facing into the vessel), secure the plug with a plastic nut, and tighten the nut. Repeat this process for each of the four plugs as needed.

8.2.3. If the plugs are already attached to the vessel, make sure that they are tightly secured or the vessel will leak.

8.2.4. Place the glass vessel onto the heat exchanger base, making sure that the vessel is centered within the bottom steel ring to prevent leaking.

8.2.5. Secure the glass vessel to the heat exchanger base by connecting the bottom steel ring to the heat exchanger base using the small thumb screws. Tighten the screws in a crosswise manner.

8.2.6. Check for leaks by pouring approximately 3L of water into the vessel and allowing it to sit for at least 5 minutes while observing the vessel for a leak.

8.2.6.1. If a leak is observed, detach the vessel and the heat exchanger base, and repeat steps 8.2.3. through 8.2.6., as needed, until no leaks are observed.

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8.2.7. If no leaks are observed, remove the water from the vessel by lifting the vessel (still attached to the heat exchanger base) and pouring the water out into a sink or container.

8.2.8. Place the baffle into the glass vessel.

8.3. **Assemble the Headplate - Underside (Figure 3)**

8.3.1. If necessary, attach the impellers to the agitator shaft. The lower impeller should be flush with the base of the agitator shaft, and the upper impeller should be approximately 6cm above the lower impeller.

8.3.2. Inspect the integrity of the O-rings on the thermowell, harvest tube and sparger tube. Replace if worn or cracked.

8.3.3. Insert the thermowell into the thermowell port from the underside of the headplate.

8.3.4. Adjust the height of the thermowell so that the base is approximately 2 cm above the lower impeller.

8.3.5. Secure the thermowell in place with a nut by lowering the nut over the top of the thermowell and hand-tightening it to the threads on the topside of the headplate.

8.3.6. Insert the harvest tube into the harvest tube port from the bottom of the headplate.

8.3.7. Adjust the height of the harvest tube so that the base is directly below the lower impeller. Rotate the lower impeller by hand to make sure that there is enough clearance for rotation without collision with the harvest tube.

8.3.8. Secure the harvest tube in place with a nut by lowering the nut over the top of the harvest tube and hand-tightening it to the threads on the topside of the headplate.

8.3.9. Insert the sparger tube into the sparger tube port from the bottom of the headplate.

8.3.10. Adjust the height of the sparger tube so that the base is directly below the lower impeller. Rotate the lower impeller by hand to make sure that there is enough clearance for rotation without collision with the sparger tube.

8.3.11. Secure the sparger tube in place with a nut by lowering the nut over the top of the sparger tube and hand-tightening it to the threads on the topside of the headplate.

8.4. Pour the cell culture media that will be used for the run into the vessel (refer to the process SOP for media compostion).

8.5. **Attach the Headplate to the Vessel**

8.5.1. Place the headplate onto the vessel.

8.5.2. Secure the headplate to the top steel ring using the large thumb screws. Tighten the screws in a crosswise manner.

8.6. **Assemble the Headplate – Top Side (Figure 4)**

8.6.1. Insert the tube of the sampler assembly into the sampler assembly port. Lower the tube until the base is approximately half way between the upper and lower impellers. Hand tighten the nut at the top of the tube.

8.6.2. Inspect the integrity of the O-ring at the bottom of the condenser. Replace if worn or cracked.

8.6.3. Insert the condenser into the condenser port and secure in place by hand-tightening the nut to the threads on the topside of the headplate. Make sure that the barbed connectors are pointing to the left. Refer to Figure 1 for proper orientation of the condenser.

8.6.4. Lower the foam probe into the foam probe port as far as it will go and then hand-tighten the nut to the threads on the topside of the headplate.

8.7. **Connect Bioreactor to Cabinet**

8.7.1. Place the vessel and heat exchanger base on the platform in front of the cabinet so that the heat exchanger base fits on the three steel plugs. The heat exchanger connectors should face the front left corner of the platform. See Figure 1 for proper orientation of the heat exchanger.

8.7.2. Make sure that the two-way valve of the sampling assembly is closed. The valve is closed when the position of the two-way valve handle is perpendicular to the sample tube, as shown in Figure 2.

8.7.3. Connect the "Jacket Water Out" water line to the top heat exchanger connector using the quick connects adaptors.

8.7.4. Connect the "Jacket Water In" water line to the bottom heat exchanger connector using the quick connects adaptors.

8.7.5. Connect the "Condenser Water Out" water line to the top condenser connector using the quick connects adaptors.

8.7.6. Connect the "Condenser Water In" water line to the bottom condenser connector using the quick connects adaptors.

8.7.7. Turn on the water. The water valve is located under the lab bench where the BioFlo 3000 is located. The pressure gauge should read between 15 and 20psi when the water is turned on.

8.8. **Calibrate the pH Probe**

8.8.1. Turn on the BioFlo 3000.

8.8.2. Select fermentation (2) and press the ENTER key.

8.8.3. Check the pH probe for any damage. Replace the probe if damaged.

8.8.4. Remove the shorting cap from the top of the pH probe and connect the pH cable to the pH probe.

8.8.5. Press the SCREEN key to select the <Calibration> screen and then press the ENTER key.

8.8.6. Remove the protective cap from the bottom of the pH probe. Carefully pour the 3M potassium chloride storage solution into a test tube with a screw cap. Label the tube as: Bioflo 3000 pH Probe Storage Solution, 3M potassium chloride. Store in a test tube rack at room temperature until needed.

8.8.7. Carefully immerse the pH and RTD temperature probes into a pH 7 standard buffer solution. Allow a few minutes for the electrode to equilibrate.

8.8.8. Use the arrow keys to select the box in the "pH" row of the "Function" column. This box should be displaying "Read" initially.

8.8.9. Change "Read" to "Zero" by pressing the ALTER key until "Zero" appears. Then press the ENTER key.

8.8.10. Use the arrow keys to select the box located in the "pH" row of the "Zero" column. Enter "7.0" using the number key pad and then press the ENTER key. Do this step even if "7.0" was already displayed when selecting the box.

8.8.11. Rinse the pH and RTD temperature probes thoroughly with deionized water. Pat them dry with a laboratory tissue.

8.8.12. Immerse the pH and RTD temperature probes into a pH 4 standard buffer solution. Allow a few minutes for the electrode to equilibrate.
8.8.13. Use the arrow keys to choose the box located in the "pH" row of the "Span" column. Enter "4.0" using the number keypad and then press the ENTER key. Do this step even if "4.0" was already displayed when selecting the box.
8.8.14. Rinse the pH and RTD temperature probes with deionized water and pat dry with a laboratory tissue.
8.8.15. Disconnect the pH cable from the pH probe, and replace the shorting cap.
8.8.16. Inspect the integrity of the internal O-ring of pH probe port. Replace if worn or cracked.
8.8.17. Apply a small amount of deionized water to the pH probe and then insert it into the pH probe port.
8.8.18. Ensure that the pH probe electrode is not touching the baffle.

8.9. **Install Dissolved Oxygen Probe**

8.9.1. Remove the protective cap from the bottom of the DO probe and inspect the screen. Replace the screen if damaged.
8.9.2. Remove the bottom housing that encases the DO probe tip.
8.9.3. Inspect the integrity of the O-ring and replace if worn or cracked.
8.9.4. Replenish the DO electrolyte in the bottom housing.
8.9.5. Replace the bottom housing.
8.9.6. Carefully insert the DO probe into the DO port of the headplate. Tighten the adaptor with a wrench.
8.9.7. Ensure that the DO probe is not touching the baffle.

8.10. **Attach Tubing**

8.10.1. Attach silicone tubing to the harvest port, bend the tube in half and secure with a clamp. Wrap glass wool and aluminum foil around the exposed end.
8.10.2. Place a piece of PharmMed tubing (approximately 1.5 meters in length) onto each addition port, bend the tubes in half and secure with clamps. Wrap glass wool and aluminum foil around the exposed ends.
8.10.3. Place silicone tubing on the top outlet of the condenser and connect an air filter. Wrap glass wool and aluminum foil around the exposed end of the filter.
8.10.4. Place silicone tubing on the top of the sparger tube and connect an air filter. Bend the tube in half and secure with a clamp. Wrap glass wool and aluminum foil around the exposed end of the tubing.
8.10.5. Remove the rubber bulb from the sampler assembly. Insert glass wool into the bulb and reattach it to the sampler assembly.

8.11. **Autoclave the vessel**

8.11.1. Turn off the BioFlo 3000.
8.11.2. Turn off the water valve (under the lab bench).
8.11.3. Disconnect the water lines to the heat exchanger.
8.11.4. Disconnect the water lines to the condenser.
8.11.5. Double check all tubes and ports to ensure that the vessel is completely sealed.
8.11.6. Autoclave the entire vessel and heat exchanger assembly for at least 30 minutes per autoclave SOP.

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8.12. **Prepare for Operation**

8.12.1. Remove the clamp from the tubing attached to the top outlet of the condenser.

8.12.2. Place the vessel and heat exchanger base on the platform in front of the cabinet so that it fits on the three steel plugs. The heat exchanger connectors should face the front left corner of the platform. See Figure 1 for proper orientation of the heat exchanger base.

8.12.3. It is advantageous to begin polarization of the DO probe at this point in the procedure. Follow directions in step 8.14.1 to do this, and then return to step 8.12.4. to connect the water lines.

8.12.4. Connect the "Jacket Water Out" water line to the top heat exchanger connector using the quick connects adaptors.

8.12.5. Connect the "Jacket Water In" water line to the bottom heat exchanger connector using the quick connects adaptors.

8.12.6. Connect the "Condenser Water Out" water line to the top condenser connector using the quick connects adaptors.

8.12.7. Connect the "Condenser Water In" water line to the bottom condenser connector using the quick connects adaptors.

8.12.8. Turn on the water. The water valve is located under the lab bench. The pressure gauge should read between 15 and 20psi.

8.12.9. Add approximately 1mL of glycerol to the thermowell and insert the RTD temperature probe into the thermowell.

8.12.10. Remove the shorting cap from the top of the pH probe and connect the pH cable to the pH probe.

8.12.11. Connect the air line from the sparger tube to the sparger gas port located on the front of the cabinet, near the top.

8.12.12. Turn on the air supply.

8.12.13. The air pressure gauge should not exceed 10psi (5psi is the ideal pressure level).

8.12.14. Remove the agitator cover from the top of the headplate and place the agitator motor securely in the agitator shaft.

8.12.15. Turn on the BioFlo 3000.

8.12.16. Select Fermentation mode (2) and press the ENTER key.

8.12.17. If the Master screen does not automatically appear use the SCREEN key to select the <Master> screen and then press the ENTER key.

8.12.18. Set the temperature control mode to "Prime" by using the arrow keys to select the box in the "Control" row of the "Temp 1" column. Press the ALTER key until "Prime" is displayed and then press the ENTER key.

8.12.19. Wait 5 minutes before setting the working temperature. Refer to the process SOP for the desired working temperature.

8.12.19.1. Set the working temperature by using the arrow keys to select the box in the "Set" row of the "Temp 1" column. Use the number keypad to enter the desired temperature and then press the ENTER key. Do this step even if the desired temperature was already displayed when selecting the box.

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8.12.20. Set the control mode to P-I-D by selecting the box in the "Control" row of the "Temp 1" column. Press ALTER until "P-I-D" appears and then press the ENTER key.
8.12.21. Set the agitation loop to P-I-D control by selecting the box in the "Control" row of the "Agit 1" column. Press the ALTER key until "P-I-D" appears and then press the ENTER key.
8.12.22. Set the agitation setpoint to 100rpm by selecting the box in the "Set" row of the "Agit 1" column. Use the number keypad to enter 100 and then press the ENTER key.
8.12.23. Set up the feed solution flasks on the appropriate feed pumps, as indicated by the process SOP. Acidic or basic solutions should be set up on Feed 1. Refer to the process SOP for indication of an acidic or basic solution.
8.12.23.1. Unclamp the tube attached to an addition port and remove the glass wool and aluminum foil.
8.12.23.2. Thread the free end of the tube through the bottom of the feed pump, counter clockwise around the pump, and out the top.
8.12.23.3. Place the free end of the tube on the top of the glass pipet of the feed solution flask.
Note: The pump moves in a clockwise direction. Therefore the solution will flow from right to left in the tube.
8.12.24. Repeat step 8.12.23 for additional feed solution flasks, if needed.
8.12.25. Set the pH setpoint to the desired value. Refer to the process SOP for the desired pH.
8.12.25.1. Set the pH setpoint by selecting the box in the "Set" row of the "pH 1" column. Use the number key pad to enter the desired pH and then press the ENTER key. Do this step even if the desired pH was already displayed when selecting the box.
8.12.26. Set the pH loop to P-I-D control by selecting the box in the "Control" row of the "pH 1" column. Press the ALTER key until "P-I-D" appears and then press the ENTER key.
8.12.27. Designate Feed 1 as acid or base. Refer to the process SOP to determine if the Feed 1 solution is acidic or basic.
8.12.27.1. Designate Feed 1 by using the arrow keys to select the box in the "Loop Name" row of the "pH 1" column. This box should be displaying the column heading "pH 1". Press the ALTER key until the "Feed 1" column heading appears then press the ENTER key.
8.12.27.2. Use the arrow keys to select the box in the "Control" row of the "Feed 1" column. Use the ALTER key to select either "Base" or "Acid" and then press the ENTER key.
8.12.27.3. Set the setpoint to 100 by choosing the box in the "Set" row of the "Feed 1" column. Use the number keypad to enter "100" and then press the ENTER key.
8.12.28. Return to the pH loop display by selecting the "Feed 1" column heading. Press the ALTER key until "pH 1" appears and then press the ENTER key.

8.12.29. Wait for the pH value to reach its setpoint before proceeding.

8.13. **Activate additional feed loops at the appropriate time as indicated by the process SOP.**

8.13.1. Activate a feed loop by selecting the box in the "Loop Name" row of the "pH 1" column. This box should be displaying the column heading "pH 1". Press the ALTER key until the "Feed 2" column heading appears (or the appropriate feed column heading if not "Feed 2").

8.13.2. Enter the appropriate setpoint for the feed solution, as indicated by the process SOP, by choosing the box in the "Set" row of the "Feed 2" column. Use the number keypad to enter the appropriate setpoint value and then press the ENTER key.

8.13.3. In the "Control" row of the "Feed 2" column press the ALTER key until "on" is displayed, then press ENTER.

8.14. **Calibrate the Dissolved Oxygen Probe**

Note: The DO probe should not be calibrated until the desired working temperature has been reached.

8.14.1. Polarize the DO probe for 30 minutes prior to performing calibration.

8.14.1.1. If necessary, connect the DO cable to the DO electrical outlet on the front of the cabinet.

8.14.1.2. Remove the protective cap from the top of the DO probe and connect the DO Cable to the DO probe.

8.14.1.3. If necessary, turn on the BioFlo 3000.

8.14.1.4. Allow the probe to sit for at least 30 minutes.

8.14.1.5. Verify that the DO level is above 0. If the DO display reads 0 or below there may be a problem with the DO probe. Contact the lab manager if this occurs.

8.14.2. Use the SCREEN key to choose the <Calibration> screen and then press the ENTER key.

8.14.3. Use the arrow keys to choose the box in the "DO" row of the "Function" column. This box should be displaying "Read".

8.14.4. Change "Read" to "Zero" by pressing the ALTER key until "Zero" appears and then pressing the ENTER key.

8.14.5. Use the arrow keys to choose the box in the "DO" row of the "Zero" column. Enter "0.0" using the number keypad and then press the ENTER key.

8.14.6. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.

8.14.7. Use the arrow keys to choose the box in the "Set" row of the "Agit 1" column.

8.14.8. Enter "1000" (for 1000rpm) using the number keypad and then press the ENTER key. This will turn on the agitation motor.

8.14.9. Press the SCREEN key and then use the arrow keys to select the <Gases> screen and then press the ENTER key.

8.14.10. Set the gases mode to "Manual" by selecting the box in the "Mode" row (it is the only box available in the "Mode" row). Press the ALTER key until "Manual" is displayed, then press the ENTER key.

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8.14.11. Allow ten to thirty minutes for the vessel to equilibrate.
8.14.12. Press the SCREEN key and then use the arrow keys to select the <Calibration> screen and then press the ENTER key.
8.14.13. Use the arrow keys to choose the box in the "DO" row of the "Function" column. This box should be displaying "Read".
8.14.14. Change "Read" to span by pressing the ALTER key until "Span" is displayed and then press the ENTER key.
8.14.15. Choose the box in the "DO" row of the "Span" column. Enter "100.0" using the number keypad and then press the ENTER key.

8.15. **There are three possible control modes for dissolved oxygen. Refer to the process SOP for the desired mode.**

8.15.1. **Controlling by Agitation Only**

8.15.1.1. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.
8.15.1.2. Set the DO loop to P-I-D by selecting the box in the "Control" row of the "DO2 1" column. Press the ALTER key until "P-I-D" appears and then press the ENTER key.
8.15.1.3. Temporarily set the agitation loop to P-I-D by selecting the box in the "Control" row of the "Agit 1" column. Press the ALTER key until "P-I-D" appears and then press the ENTER key.
8.15.1.4. To set the minimum rpm select the box in the "Set" row of the "Agit 1" column. Use the number keypad to enter the desired minimum rpm, as indicated by the process SOP and then press the ENTER key.
8.15.1.5. Set the agitation loop to DO control mode by selecting the box in the "Control" row of the "Agit 1" column. Press the ALTER key until "DO" appears and then press the ENTER key.
8.15.1.6. To set the maximum rpm select the box in the "Set" row of the "Agit 1" column. Use the number keypad to enter the desired maximum rpm, as indicated by the process SOP and then press the ENTER key.
Note: The controller will automatically adjust the rpm, therefore after the maximum value is entered the displayed set value may change immediately afterward.
8.15.1.7. To input the DO setpoint select the box in the "Set" row of the "DO2 1" column. Use the number keypad to enter the desired setpoint, as indicated by the process SOP and then press the ENTER key.

8.15.2. **Controlling by % Oxygen Sparged**

8.15.2.1. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.
8.15.2.2. Set the DO loop to P-I-D by selecting the box in the "Control" row of the "DO2 1" column. Press the ALTER key until "P-I-D" appears and then press the ENTER key.
8.15.2.3. Press the SCREEN key and then use the arrow keys to choose the <Gases> screen and then press the ENTER key.

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8.15.2.4. Set the gases mode to DO control by selecting the box in the "Mode" row (it is the only box available in the "Mode" row). Press the ALTER key until "DO" is displayed and then press the ENTER key.

8.15.2.5. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.

8.15.2.6. To input the DO setpoint select the box in the "Set" row of the "DO2 1" column. Use the number keypad to enter the desired setpoint, as indicated by the process SOP and then press the ENTER key.

8.15.3. **Controlling by Agitation and % Oxygen**

Note: In this mode agitation will be increased first until maximum rpm is reached and more oxygen is required, then control by % oxygen will begin.

8.15.3.1. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.

8.15.3.2. Set the DO loop to P-I-D by selecting the box in the "Control" row of the "DO2 1" column. Press the ALTER key until "P-I-D" appears and then press the ENTER key.

8.15.3.3. Temporarily set the agitation loop to P-I-D by selecting the box in the "Control" row of the "Agit 1" column. Press the ALTER key until "P-I-D" appears and then press the ENTER key.

8.15.3.4. To set the minimum rpm select the box in the "Set" row of the "Agit 1" column. Use the number keypad to enter the desired minimum rpm, as indicated by the process SOP and then press the ENTER key.

8.15.3.5. Set the agitation loop to DO control mode by selecting the box in the "Control" row of the "Agit 1" column. Press the ALTER key until "DO" appears and then press the ENTER key.

8.15.3.6. To set the maximum rpm select the box in the "Set" row of the "Agit 1" column. Use the number keypad to enter the desired maximum rpm, as indicated by the process SOP and then press the ENTER key.
Note: The controller will automatically adjust the rpm, therefore after the maximum value is entered the displayed set value may change immediately afterward

8.15.3.7. Press the SCREEN key and then use the arrow keys to choose the <Gases> screen and then press the ENTER key.

8.15.3.8. Set the gases mode to agitation and % oxygen control by selecting the box in the "Mode" row (it is the only box available in the "Mode" row). Press the ALTER key until "Ag02DO" is displayed and then press the ENTER key.

8.15.3.9. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.

8.15.3.10. Navigate to the air monitor loop by selecting the column heading "Agit 1" in the "Loop Name" row. Press the ALTER key until "Air 1" appears.

8.15.3.11. Input the desired air pressure by selecting the box in the "Set" row of the "Air 1" column. Use the number keypad to enter the desired air flow rate (usually between 1.5 and 2 L/min.) and then press the ENTER key.

8.15.3.12. Return to the agitation display by selecting the "Air 1" column heading. Press the ALTER key until "Agit 1" appears and then press the ENTER key.

8.15.3.13. To input the DO setpoint select the box in the "Set" row of the "DO2 1" column. Use the number keypad to enter the desired setpoint, as indicated by the process SOP and then press the ENTER key.

8.16. **Use of Foam Sensor**

8.16.1. Set up an autoclaved feed solution flask containing an antifoam agent according to the process SOP.

8.16.2. Set up the feed solution flask containing and antifoam agent on Feed 2 following directions in step 8.12.23.

8.16.3. Connect the foam sensor cable (black) to the Level 1 connector on the front of the cabinet.

8.16.4. Connect the foam sensor cable to the foam sensor.

8.16.5. Connect the red cable to the ground stud located on top of the headplate.

8.16.6. Use the SCREEN key to select the <Master> screen and then press the ENTER key.

8.16.7. Use the arrow keys to select the "Agit 1" column heading. Press the ALTER key until the "Feed 2" column heading appears and then press the ENTER key.

8.16.8. Use the arrow keys to select the box in the "Control" row of the "Feed 2" column.

8.16.9. Set the control mode to Level 1 by pressing the ALTER key until "Lvl 1" appears and then press the ENTER key.

8.16.10. Determine the foam percentage setpoint at which the antifoam agent should be added by referring to the process SOP.

8.16.11. Input the desired foam percentage setpoint by selecting the box in the "Set" row of the "Feed 2" column. Use the number keypad to enter the desired setpoing and then press the ENTER key.

8.16.12. Select the "Feed 2" column heading. Press the ALTER key until "Lvl 1" appears and then press the ENTER key.

8.16.13. Set the Level 1 control mode to add by selecting the box in the "Control" row of the "Lvl 1" column. Press the ALTER key until "On" appears and then press the ENTER key.

8.16.13.1. Return to the agitation display by selecting the "Lvl 1" column heading. Press the ALTER key until "Agit 1" appears and then press the ENTER key.

8.17. **Fermentation Procedure**

8.17.1. At this point we are ready to do the final preparation before inoculation. Our fermentation run is going to be monitored and controlled by a computer using the BioCommand Lite Software.

8.17.2. Check that the personal computer is connected to the BioFlo 3000 cabinet. If they are not connected, request that the lab manager or course instructor connect the computer to the cabinet before proceeding.

8.17.3. Turn on the computer.

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8.17.4. From the desktop open BioCommand Lite by double clicking on the BCLite icon.

8.17.5. You can choose to use an existing recipe or create a new recipe. To use an existing recipe follow the procedure in step 8.17.6. To create a new recipe follow the procedure in step 8.17.7.

8.17.6. To use an **existing recipe**, choose "Open an Existing Recipe" and then click "Continue".

8.17.6.1. Select the appropriate recipe (i.e., Pichia Master 1.rcp).

8.17.6.2. Verify that data is collected at 60min intervals in the "Time Data" tab under "Log Interval (Mins)". If the Log Interval is not 60, change it to 60 using the following procedure.

8.17.6.2.1. Click on "Change Interval"

8.17.6.2.2. Select "60" from the pull down menu.

8.17.6.2.3. Click "Save".

8.17.6.3. Click on the "Views" tab, and double click "Trend.3" in the column on the left under "Current Views".

8.17.6.4. Verify that the Trend.3 ranges are appropriate per the process SOP.

8.17.6.5. On the main screen, click "Start Batch".

8.17.6.6. Save batch file as the product lot number (i.e. 21Feb08HSA).

8.17.6.7. Skip step 8.17.7. and continue with the SOP at step 8.17.8.

8.17.7. To create a **new recipe**, from the "BioCommand Lite" window select "Create a new recipe with Batch Wizard" and click "Continue".

8.17.7.1. In the "BioCommand Batch Wizard-Introduction" window click "Next".

8.17.7.2. In the "BioCommand Batch Wizard –Step 1" window type in "Recipe1" under "Enter Recipe Display Name:" and click "Next".

8.17.7.3. In the "BioCommand Batch Wizard-Step 2" window click "Select Loops".

8.17.7.4. Click on "Browse Local Server".

8.17.7.5. Under "Available Loops:" highlight "BIOLAB01" then click ">>". This adds "BIOLAB01" to the "Loops in Batch" box.

8.17.7.6. Under "Available Loops:" highlight "Controller 1" and click "Browse Local Server."

8.17.7.7. After the + sign appears next to "Controller 1" click ">>".

8.17.7.8. Click "Close".

8.17.7.9. When the "Biocommand Batch Wizard –Step 2" window reappears, click "Next".

8.17.7.10. The "Biocommand Batch Wizard –Step 3" window should appear with the recipe file name. File name format should be: "C:\BIOCOM~1\BIORcp[#].rcp". Click "Next".

8.17.7.11. In the "Biocommand Batch Wizard –Step 4" window click "Next".

8.17.7.12. In the "Biocommand Batch Wizard – Finished!" window, click "Finish".

8.17.7.13. In the "Recipe[#].B1:Setup.[#]" window, select "Start Batch".

8.17.7.14. If needed, type in "Recipe1" in the "Batch Display Name" window. ("Recipe1" may automatically appear.). Click "OK".

8.17.7.15. In the "Biocommand Save Files" window save batch file as:

Title: New Brunswick BioFlo 3000 Bioreactor SOP

BT220 [semester] [year]. Record file name in the batch record, then click "OK".

8.17.7.16. From the "Recipe[#].B1:Setup.[#]" window, click on the "Views" tab.
8.17.7.17. In the "New Views:" box, double click on "Trend".
8.17.7.18. The "Recipe[#].B1:Trend.[#]" window should appear.
8.17.7.19. Display trend data using the following procedure:
8.17.7.19.1. From the "Recipe[#].B1:Trend.[#]" window, select the "Loops" tab.
8.17.7.19.2. Click on "Setup Loop 1" (or the appropriate Loop number).
8.17.7.19.3. From the "Select Loop" window, highlight one of the parameters to be monitored (i.e.: Agit 1). Then click "OK". The parameter name chosen is automatically transferred to the "Recipe[#].B1:Trend.[#]" window.
8.17.7.19.4. Click ">>".
8.17.7.19.5. Click "Setup Loop2".
8.17.7.19.6. From the "Select Loop" window, highlight another parameter.
8.17.7.19.7. Click "OK".
8.17.7.19.8. Click ">>".
8.17.7.20. Repeat step 8.17.26. until all parameters needed are added to the "Recipe[#].B1:Trend.[#]" window. (AGIT1, DO2 1, TEMP 1 and pH 1 should always be added.)
8.17.7.21. Set the graph range for each parameter using the following procedure.
8.17.7.21.1. In the "Recipe[#].B1:Trend.[#]" window select the "Graphs" tab. Use "<<" to navigate all the way back to the first parameter (Loop 1). Enter the values listed below into the appropriate box for each parameter then select ">>" to apply the change to the graph.

Parameter	Low:	High:
AGIT1	100	1100
DO21	0.0	100
TEMP1	0.0	40.0
pH1	1.0	7.0
Feed1	0	10
Feed 2	0	10

8.17.8. **Do NOT close any windows during a run.**

8.18. **Inoculation Procedure**
8.18.1. Allow the bioreactor time to reach all of its setpoints.
8.18.2. Attach the bulb to sample port (be sure there is glass wool in tube before attaching).
8.18.3. Verify that all of the setpoint parameters are within range and then aseptically add the inoculum through the inoculation port per the process SOP.
8.18.4. Take immediate sample per process SOP for baseline determination.

8.19. **Monitor Parameters per process SOP.**
8.20. **Harvest Culture per process SOP.**
8.21. **Shut Down Procedure**

Title: New Brunswick BioFlo 3000 Bioreactor SOP

8.21.1. Select "End Batch."
8.21.2. Exit BCLite program.
8.21.3. On the desktop, select DBViewer32.
8.21.4. Select browse and choose the file used during the run.
8.21.5. Export the data to Microsoft Excel.
8.21.6. Turn all the control modes (Temp, pH, Agit, DO, Feed 1-5) on the Bioflo 3000 controller to off.
8.21.7. Turn off the BioFlo 3000.
8.21.8. Turn off the water flow.

8.22. **Clean the BioFlo 3000.**

8.22.1. Make up approximately 4 liters of 10% bleach solution. Label each vessel containing the bleach solution as: 10% Bleach, [date], [initials], Store: Room Temperature, Dispose: Drain.
8.22.2. Disconnect all cords and tubes on the outside of the vessel assembly.
8.22.3. Pour 3L of 10% bleach solution into the vessel through the inoculation port.
8.22.4. Soak the vessel in the 10% bleach solution for approximately 15 minutes.
8.22.5. Remove the agitator motor and wipe down with a sponge using 10% bleach solution.
8.22.6. Remove the headplate from the vessel.
8.22.7. Remove the baffle from the vessel. Clean the baffle with water and glassware detergent. Scrub clean with a soft brush if needed. Rinse thoroughly with tap water and then with deionized water.
8.22.8. Remove the vessel from the heat exchanger base and pour the bleach solution down the drain. Rinse the vessel thoroughly.
8.22.9. Remove the plastic plugs from the vessel.
8.22.10. Clean the vessel carefully with warm water, glassware detergent and a sponge. Rinse thoroughly with tap water and then deionized water.
Note: Handle the vessel gently, the glass is very brittle.
8.22.11. Clean the plastic plugs and thumb screws (large and small) thoroughly with warm water and glassware detergent. Scrub clean with a soft brush if needed. Rinse thoroughly with tap water and then with deionized water.
8.22.12. Remove all probes and tubes from the topside and underside of the headplate.
8.22.13. Place DO and pH probes securely in the probe stand and place the shorting caps on the top of each probe.
8.22.14. Clean all bioreactor tubes with a soft cloth, warm water, and glassware detergent. Rinse thoroughly with warm tap water, and then with several changes of deionized water.
Note: A soft brush may be used on difficult to clean parts of the bioreactor vessel.
8.22.15. Clean the inside of the sparger tube to remove clogs and prevent damage to the bioreactor on a future run.
8.22.16. Remove the pH and DO probes one at a time from the probe stand and gently clean the probes using a soft cloth and deionized water. Be careful not to harm

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the sensory tips of these probes. Return each probe to the probe stand after cleaning.

8.22.17. Add pH probe storage solution (3M potassium chloride) to the protective cover of the pH probe. The cover should be approximately half full of solution.

8.22.18. Carefully cover the pH probe with the protective cap. The probe should be immersed in pH probe storage solution.

8.22.19. Place the DO probe protective cap on the bottom of the DO probe.

8.22.20. DO and pH probes should be stored in an upright position in the probe stand.

8.22.21. Clean the headplate and impellers with water and glassware detergent. A soft brush or sponge can be used. Rinse thoroughly with tap water and then deionized water.

8.22.22. Remove the O-ring from the headplate and heat exchanger base and clean thoroughly.

8.22.23. Clean the heat-exchanger base and rinse thoroughly.

8.22.24. Ensure that all vessel parts have been cleaned and rinsed thoroughly. Allow them to air dry.

8.22.25. Wipe down the BioFlo cabinet with 10% bleach solution.

9. Attachments:

9.1. Figure 1: BioFlo 3000 Vessel and Cabinet
9.2. Figure 2: Vessel Parts
9.3. Figure 3: Headplate, Underside
9.4. Figure 4: Assembled Headplate, Top Side
9.5. Figure 5: BioFlo 3000 Side View
9.6. Figure 6: BioFlo 3000 Controller

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial release
Sue Penney	2003	Updated.
Karl J. Cresswell	121905	Removed all grease steps and added inspection of DO probe.
Deb Audino	20July07	Added Feed 1 and 2 low and high values. Added inputing DO setpoint.
Deb Audino	04Apr08	College name change. Added steps for using an existing recipe.
Kari Britt	15May09	Changed directions for calibration of the pH meter and dissolved oxygen probes. Changed directions in “Prepare for Operation” section. Changed directions for assembling the headplate. Changed directions for the three types of control modes. Clarified directions for using BCLite. Removed figures and added additional figures in the attachments section.

Title: New Brunswick BioFlo 3000 Bioreactor SOP

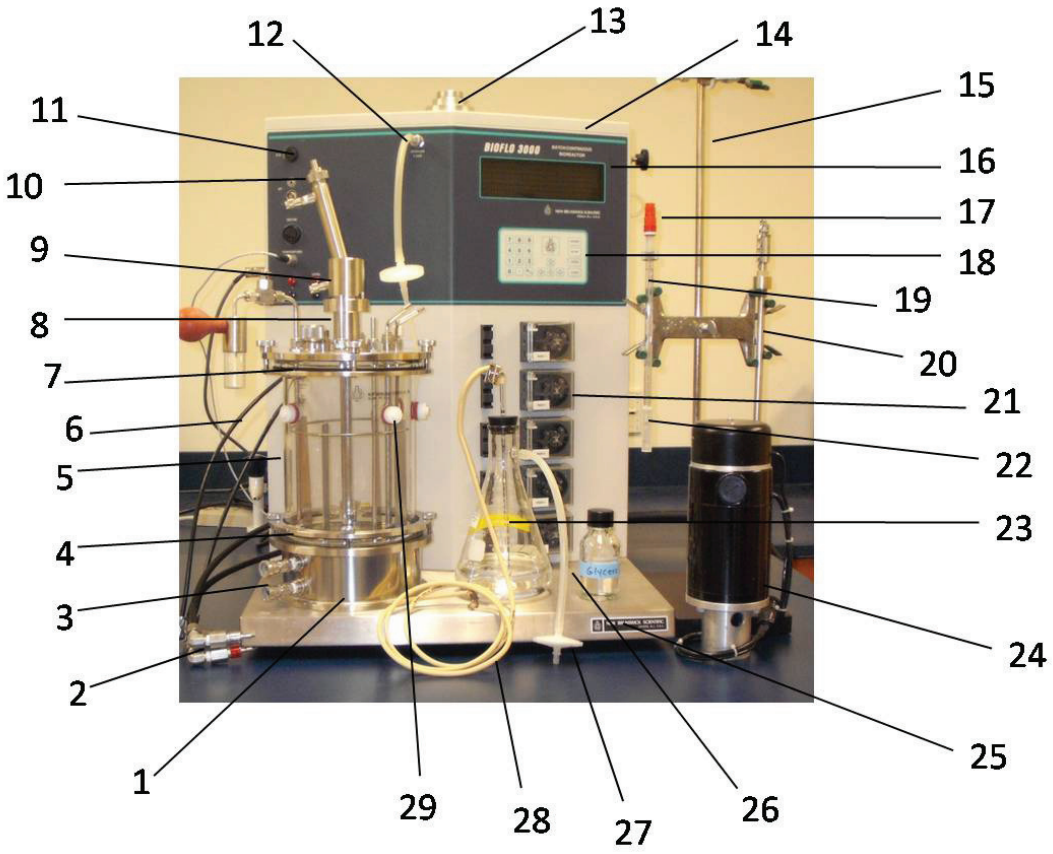


Figure 1: BioFlo 3000 Vessel and Cabinet

1. Heat Exchanger Base
2. Jacket Water Line Adaptors
3. Heat Exchanger Connectors
4. Bottom Steel Ring
5. Glass Vessel
6. Condenser Water Lines
7. Top Steel Ring
8. Agitator Shaft
9. Agitator Cover
10. Condenser Top Outlet
11. Electrical Outlet
12. Sparger Gas Port
13. Agitator Motor Stand
14. Cabinet
15. Probe Stand
16. Controller Display
17. pH Probe Shorting Cap
18. Controller Keypad
19. pH Probe
20. DO Probe
21. Feed Pump
22. pH Probe Protective Cap
23. Feed Bottle Assembly
24. Agitator Motor
25. Platform
26. Silicone Tubing
27. Air Filter
28. PharMed Tubing
29. Plastic Plug

Title: New Brunswick BioFlo 3000 Bioreactor SOP

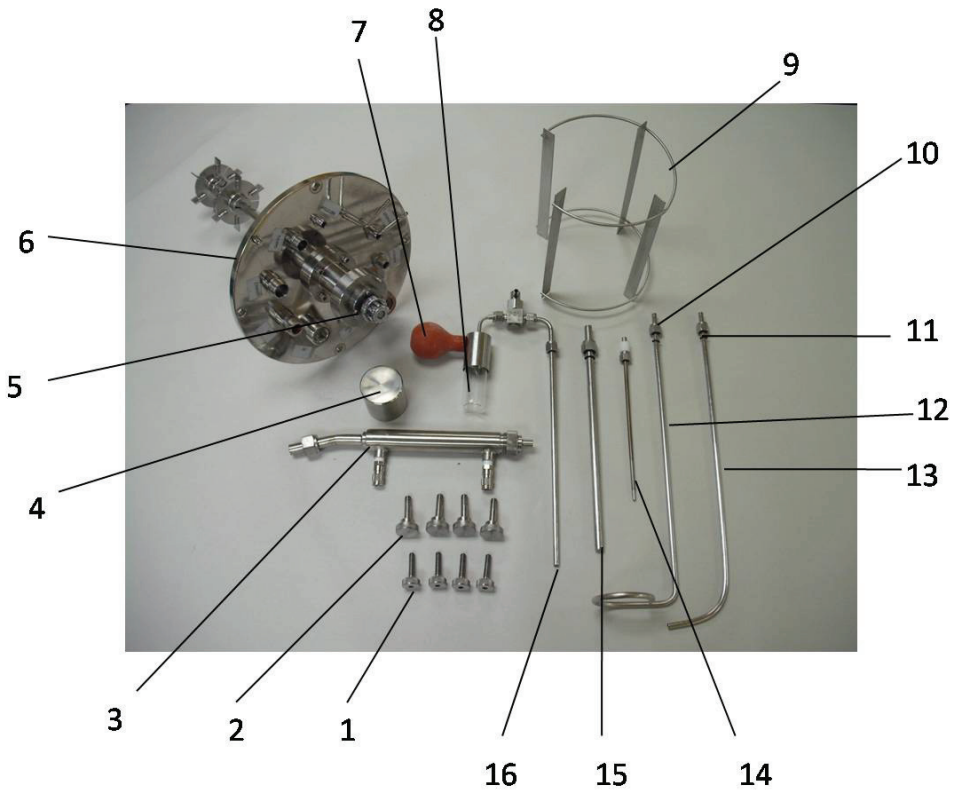


Figure 2: Vessel Parts

1. Small Thumb Screw
2. Large Thumb Screw
3. Condenser
4. Agitator Cover
5. Agitator
6. Headplate
7. Sampler Assembly Bulb
8. Sample Bottler
9. Baffle
10. Nut
11. O-ring
12. Harvest Tube
13. Sparger Tube
14. Foam Sensor
15. Thermowell
16. Sampler Assembly

Title: New Brunswick BioFlo 3000 Bioreactor SOP

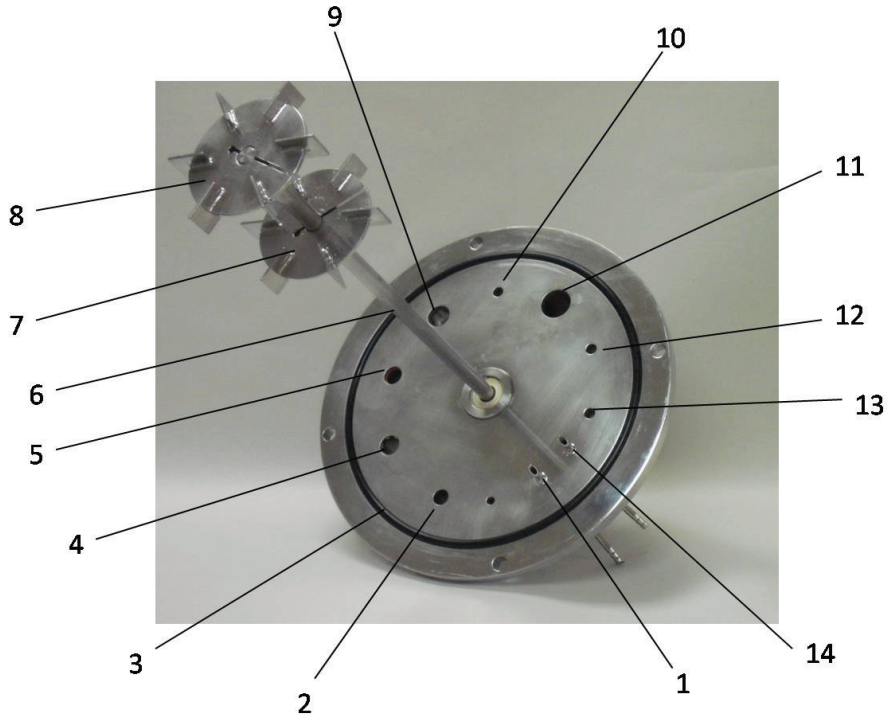


Figure 3: Headplate, Underside

1. Addition Port
2. Thermowell Port
3. O-ring
4. Foam Sensor Port
5. pH Probe Port
6. Agitator Shaft
7. Upper Impeller
8. Lower Impeller
9. DO Probe Port
10. Sampler Assembly Port
11. Inoculation Port
12. Harvest Tube Port
13. Sparger Tube Port
14. Addition Port

Title: New Brunswick BioFlo 3000 Bioreactor SOP

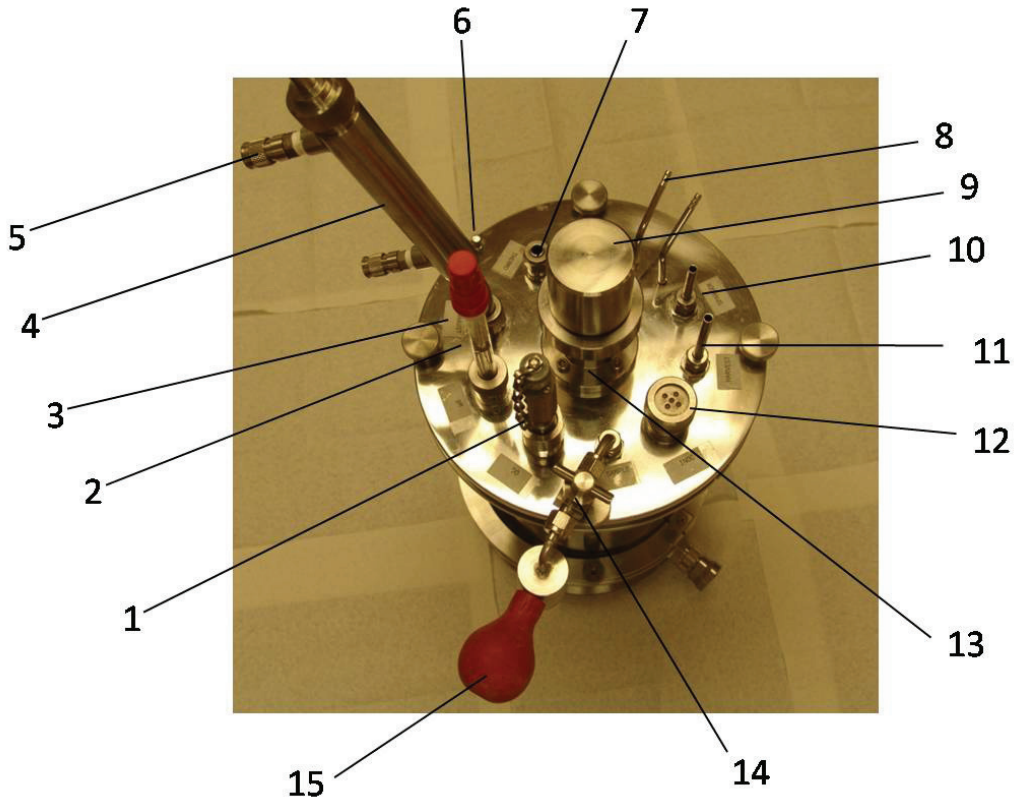


Figure 4: Assembled Headplate, Top Side

1. DO Probe
2. pH Probe
3. Foam Sensor (behind pH Probe)
4. Condenser
5. Condenser Barbed Connectors
6. Ground Stud
7. Thermowell
8. Addition Port
9. Agitator Cover
10. Sparger Tube
11. Harvest Tube
12. Inoculation Port
13. Agitator Shaft
14. Sampler Assembly Valve (in closed position)
15. Sampler Assembly Bulb.

Title: New Brunswick BioFlo 3000 Bioreactor SOP

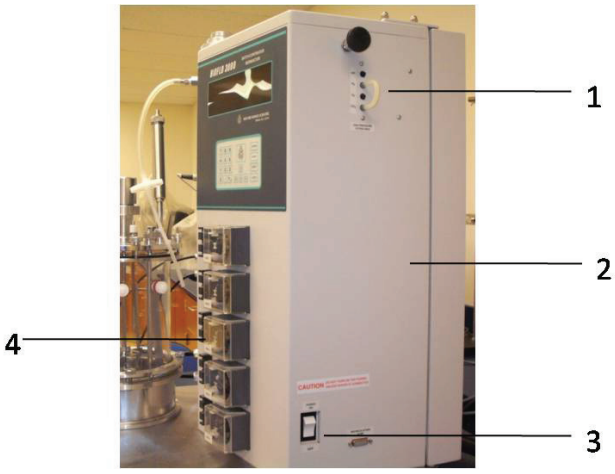


Figure 5: BioFlo 3000 Side View

1. Gas and Air Console
2. Cabinet
3. Power Switch
4. Feed Pump

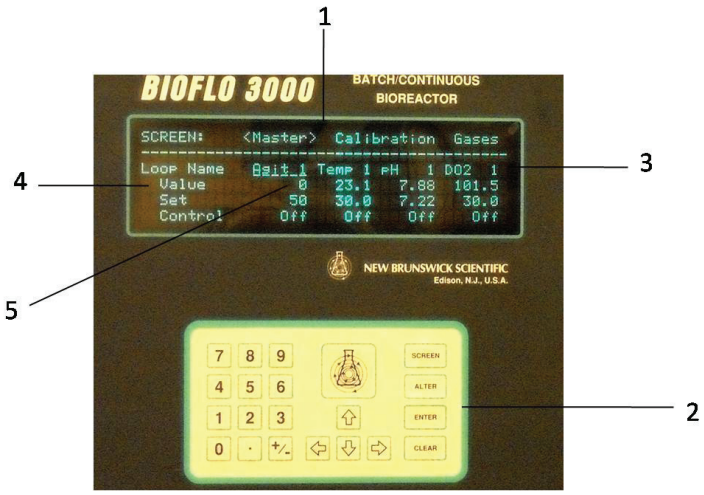


Figure 6: BioFlo 3000 Controller

1. Display Screen
2. Keypad
3. Column Heading
4. Row Name
5. Box (Where the Column Heading and Row Name coordinates meet.)

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

Record Keeping Standards:

For each step in the batch record: the operator of the task will enter their initials (each operator has their own unique set of initials) and the date in the appropriate section(s) of the batch record. Another operator must initial and date in the appropriate section of the batch record to verify that the task was completed per SOP. No operator will verify their own work at any point. "If you didn't document it, you didn't do it!"

Batch records will be completed in blue or black ball point pen ONLY, and must be legible.

Any errors on a batch record will be crossed out with a single line through the error with the initials of the operator and the date. Corrections will be written in next to the crossed out error.

Use the following format to record dates: DDMMMYY. For July 10, 2006 use 10JUL06.

Use the 24 hour clock or "military time" to record time: 3:00pm would be written as 15:00.

Any and all deviations from a protocol or SOP, including abnormal results or retests performed, will be entered into the comments section at the end of each batch record. Be as detailed and specific as possible, include all steps taken before and/or after an abnormal reading, and provide an explanation for any deviations from a step.

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

1. Media Preparation for Seed Flask Cultures		
Dissolve 1.3 ± 0.05g K_2HPO_4 and 5.8 ± 0.05g KH_2PO_4 into 500 ± 5mL of deionized water in a 1L flask. K_2HPO_4 (potassium phosphate dibasic anhydrous) Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams KH_2PO_4 (potassium phosphate monobasic anhydrous) Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams Volume of water added: _____mL	Operator/Date	Verifier/Date
Adjust 0.1M potassium phosphate buffer to pH 6±0.1. pH_____	Operator/Date	Verifier/Date
Add 5±0.5g yeast extract to the potassium phosphate buffer. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Add 10±0.5g peptone to the potassium phosphate buffer. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Add 10 ±0.5g glucose to the potassium phosphate buffer. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____________________

Transfer 90mL of the 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone into a 500mL shake flask. Shake Flask ID:___________ Volume of media transferred ____________ mL	Operator/Date	Verifier/Date
Transfer 90mL of the 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone into a 500mL shake flask. Shake Flask ID:___________ Volume of media transferred: ____________ mL	Operator/Date	Verifier/Date
Transfer 90mL of the 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone into a 500mL shake flask. Shake Flask ID:___________ Volume of media transferred: ____________ mL	Operator/Date	Verifier/Date
Transfer 90mL of the 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone into a 500mL shake flask. Shake Flask ID:___________ Volume of media transferred: ____________ mL	Operator/Date	Verifier/Date
Transfer 90mL of the 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone into a 500mL shake flask. for use in cryopreservation. Shake Flask ID:___________ Volume of media transferred: ____________ mL	Operator/Date	Verifier/Date
Transfer 36mL of the media into a 100mL bottle for blanking the spectrophotometer. Volume of media transferred ____________ mL	Operator/Date	Verifier/Date
Autoclave 500mL flasks and 100mL bottle of media per autoclave SOP. Autoclave ID:______________________	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number____________________

Prepare 10x YNB Solution: Weigh out 6.7±0.02g yeast nitrogen base without amino acids and combine with 100±1mL deionized water. Manufacturer:_______________Catalog number: _______________ Lot number: _______________Expiration date:_______________ Amount weighed:_______________grams Volume of water added: _________________mL	Operator/Date	Verifier/Date
Filter sterilize the 10X YNB and label as: Sterile Filtered 10X YNB, [date], [initials], Store: 2-8°C, Dispose: drain.	Operator/Date	Verifier/Date
Aseptically add 10mL 10X YNB to the COOLED autoclaved flask of media containing 90mL of media. Shake Flask I.D.: _________ Group:__________ Volume of 10X YNB added to flask: ___________	Operator/Date	Verifier/Date
Aseptically add 10mL 10X YNB to the COOLED autoclaved flask of media containing 90mL of media. Shake Flask I.D.: _________ Group:__________ Volume of 10X YNB added to flask: ___________	Operator/Date	Verifier/Date
Aseptically add 10mL 10X YNB to the COOLED autoclaved flask of media containing 90mL of media. Shake Flask I.D.: _________ Group:__________ Volume of 10X YNB added to flask: ___________	Operator/Date	Verifier/Date
Aseptically add 10mL 10X YNB to the COOLED autoclaved flask of media containing 90mL of media. Shake Flask I.D.: _________ Group:__________ Volume of 10X YNB added to flask: ___________	Operator/Date	Verifier/Date
Aseptically add 10mL 10X YNB to the COOLED autoclaved flask of media containing 90mL of media for the purpose of cryopreservation. Shake Flask I.D.: _________ Volume of 10X YNB added to flask: ___________	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

Aseptically add 4mL 10X YNB to the COOLED autoclaved 100mL glass bottle containing 36mL of media. Volume of 10X YNB added to 100mL bottle: _____	Operator/Date	Verifier/Date
Label the five shake flasks as: 0.1M Potassium Phosphate Media, pH 6, 1X YNB, with 1% Yeast Extract and 2% Peptone, [date], [group], [initials], Store: 2-8°C, Dispose: drain	Operator/Date	Verifier/Date
Label the 100mL bottle as: 0.1M Potassium Phosphate Media, pH 6, 1X YNB, with 1% Yeast Extract and 2% Peptone, [date], [initials], Blanking Media for Spectrophotometer, Store: 2-8°C, Dispose: drain.	Operator/Date	Verifier/Date
Proof the media in the shake flasks at 37 ± 0.5 °C shaking at approximately 200 rpm for a minimum of 24 hours. Incubation Time:_____	Operator/Date	Verifier/Date
Check media for contamination. If contaminated, add bleach and dispose down drain. Shake Flask I.D.: _____ Group:_____ Contamination: YES/NO (Circle one) Bleached and disposed down drain: YES/NO (Circle one)	Operator/Date	Verifier/Date
Check media for contamination. If contaminated, add bleach and dispose down drain. Shake Flask I.D.: _____ Group:_____ Contamination: YES/NO (Circle one)	Operator/Date	Verifier/Date
Check media for contamination. If contaminated, add bleach and dispose down drain. Shake Flask I.D.: _____ Group:_____ Contamination: YES/NO (Circle one)	Operator/Date	Verifier/Date
Check media for contamination. If contaminated, add bleach and dispose down drain. Shake Flask I.D.: _____ Group:_____ Contamination: YES/NO (Circle one)	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

Check media intended for cryopreservation for contamination. If contaminated, add bleach and dispose down drain. Shake Flask I.D.: _____ Contamination: YES/NO (Circle one)	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
2. Seed Flask Culture		
Thaw contents of 1mL cryovials (one vial per shake media) of *Pichia pastoris* cells in 30°C water bath. Shake Flask ID _____Vial ID _____ Shake Flask ID _____Vial ID _____ Shake Flask ID _____Vial ID _____ Shake Flask ID _____Vial ID _____	Operator/Date	Verifier/Date
Prepare the biological safety cabinet (BSC) per the BSC SOP.	Operator/Date	Verifier/Date
Sterilely transfer the contents of each vial to a flask containing 100mL media. Label flasks as Pichia Inoculum, [group], [date], [initials], Dispose: Autoclave then drain.	Operator/Date	Verifier/Date
Incubate flasks 24-48 hours in shaking incubator at 30°C at approx. 200 rpm.	Operator/Date	Verifier/Date
Aseptically remove a 2mL sample from each seed flask and place into a corresponding labeled cuvette. Take OD reading of cultures at 600nm. Shake Flask ID_____ Group_____ OD_____ Shake Flask ID_____ Group_____ OD_____ Shake Flask ID_____ Group_____ OD_____ Shake Flask ID_____ Group_____ OD_____	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

Prepare a Gram stain of each culture per the Gram Stain SOP. Examine for contamination of cultures. Shake Flask ID_____ Group_____ Contamination: YES/NO (Circle one) Shake Flask ID_____ Group_____ Contamination: YES/NO (Circle one) Shake Flask ID_____ Group_____ Contamination: YES/NO (Circle one) Shake Flask ID_____ Group_____ Contamination: YES/NO (Circle one)	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
3. Media Preparation for Bioreactor		
Dissolve 2.3 ± 0.05g K_2HPO_4 and 10.4 ± 0.05g KH_2PO_4 in 900 ± 10mL of deionized water in a 2L flask. K_2HPO_4 (potassium phosphate dibasic anhydrous) Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams KH_2PO_4 (potassium phosphate monobasic anhydrous) Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams Volume of water added: _____mL	Operator/Date	Verifier/Date
Add 20 ± 0.5 grams glucose to the media. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Adjust 0.1M potassium phosphate buffer to pH 6 ±0.1. pH_____	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

Label flask as: 0.1M Potassium Phosphate Media, pH 6, [date], [initials], Store: 2-8°C, Dispose: drain.	Operator/Date	Verifier/Date
Dissolve 2.3 ± 0.05g K_2HPO_4 and 10.4 ± 0.05g KH_2PO_4 into 900 ± 10mL of deionized water in a 2L flask. K_2HPO_4 (potassium phosphate dibasic anhydrous) Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams KH_2PO_4 (potassium phosphate monobasic anhydrous) Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams Volume of water added: _____mL	Operator/Date	Verifier/Date
Add 20 ± 0.5 grams glucose to the media. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Adjust 0.1M potassium phosphate buffer to pH 6 ±0.1. pH_____	Operator/Date	Verifier/Date
Label flask as: 0.1M Potassium Phosphate Media, pH 6, [date], [initials], Store: 2-8°C, Dispose: drain.	Operator/Date	Verifier/Date
Dissolve 2.3 ± 0.05g K_2HPO_4 and 10.4 ± 0.05g KH_2PO_4 into 900 ± 10mL of deionized water in a 2L flask. K_2HPO_4 (potassium phosphate dibasic anhydrous) Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams KH_2PO_4 (potassium phosphate monobasic anhydrous) Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams Volume of water added: _____mL	Operator/Date	Verifier/Date
Add 20 ± 0.5 grams glucose to the media. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

Adjust 0.1M potassium phosphate buffer to pH 6 ±0.1. pH_____	Operator/Date	Verifier/Date
Label flask as: 0.1M Potassium Phosphate Media, pH 6, [date], [initials], Store: 2-8°C, Dispose: drain.	Operator/Date	Verifier/Date
Prepare 300mL 10x YNB Solution: Weigh out 20.1±0.05g yeast nitrogen base without amino acids and combine with 300±5mL deionized water. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams Volume of water added: _____mL	Operator/Date	Verifier/Date
Filter sterilize the 10X YNB and label as: Sterile Filtered 10X YNB, [date], [initials], Store: 2-8°C, Dispose: drain	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
4. Assemble BioFlo 3000 per BioFlo 3000 SOP		
Clean all bioreactor parts per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Assemble the vessel per the BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Assemble the headplate (underside) per the BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Aseptically add 2.7L of 0.1M Potassium Phosphate Media, pH 6 to the vessel per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Attach the headplate to the vessel per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Assemble the headplate (top side) per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Connect the bioreactor to the cabinet per the BioFlo 3000 SOP.	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

Calibrate the pH probe per the BioFlo 3000 SOP using commercially prepared standard buffers (pH 7 and pH 4): pH 7 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ pH 4 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Apply a small amount of deionized water to the pH probe and then insert it into the pH probe port.	Operator/Date	Verifier/Date
Ensure that the pH probe is not touching the baffle.	Operator/Date	Verifier/Date
Install dissolved oxygen probe per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Remove protective cap from the bottom of the DO probe and inspect screen. Replace if damaged. Protective screen damaged? Yes / No (Circle one.) Protective screen replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Unscrew the bottom housing of the probe tip. Inspect the integrity of the O-ring. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Replenish DO electrolyte.	Operator/Date	Verifier/Date
Carefully insert the DO probe into the DO port of the headplate.	Operator/Date	Verifier/Date
Ensure that the DO probe is not touching the baffle.	Operator/Date	Verifier/Date
Attach tubing per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Autoclave the entire assembly at a minimum of 121°C for at least 30 minutes per BioFlo 3000 SOP and autoclave SOP.	Operator/Date	Verifier/Date
Aseptically add 300mL of filtered 10X YNB through the inoculation port.	Operator/Date	Verifier/Date
5. Prepare Feed Solutions for BioFlo 3000		
Assemble two 1L flasks (each with a sidearm) for feed solutions per process SOP.	Operator/Date	Verifier/Date
Autoclave the two assembled 1L flasks per autoclave SOP.	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

Aseptically pour approximately 500mL of 30% NH_4OH into an assembled feed solution flask. CAUTION: Wear safety glasses and pour in a fume hood.	Operator/Date	Verifier/Date
Aseptically pour approximately 1L of 100% methanol into an assembled feed solution flask.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
6. Prepare Bioreactor for Operation		
Prepare the BioFlo 3000 for operation per the BioFlo 3000 SOP.	Operator/Date	Verifier/Date
When prompted by the BioFlo 3000 SOP, input the working temperature into the control panel of the bioreactor. Desired Working Temperature: 30°C	Operator/Date	Verifier/Date
Set up the 1L flask containing 30% NH_4OH solution on Feed 1 per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Set up the 1L flask containing 100% methanol solution on Feed 2 per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
When prompted by the BioFlo 3000 SOP, input the desired pH into the control panel of the bioreactor. Desired pH: 6.0	Operator/Date	Verifier/Date
Calibrate the dissolved oxygen probe per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Set DO mode to control by agitation only per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Set the minimum agitation rpm to 200.	Operator/Date	Verifier/Date
Set the maximum agitation rpm to 1000.	Operator/Date	Verifier/Date
Set the dissolved oxygen (DO) level to 30%.	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

Comments:	Operator/Date	Verifier/Date
7. Fermentation Procedure		
Set up and start the BioCommand Lite program according to the instructions in the Fermentation Procedure section of the BioFlo3000 SOP.	Operator/Date	Verifier/Date
Record Biocommand Lite File name:	Operator/Date	Verifier/Date
8. Inoculation Procedure		
Verify that the bioreactor has reached all of its setpoints and that the setpoint parameters are within range before inoculation.	Operator/Date	Verifier/Date
Choose the seed culture(s) that has the highest OD and has NO contamination to inoculate the BioFlo 3000. Record which shake flask(s) was used to inoculate bioreactor below: Shake Flask ID_____ OD_____ Group _____ Contamination: YES/NO (Circle one) Shake Flask ID_____ OD_____ Group _____ Contamination: YES/NO (Circle one) Shake Flask ID_____ OD_____ Group _____ Contamination: YES/NO (Circle one) Shake Flask ID_____ OD_____ Group _____ Contamination: YES/NO (Circle one)	Operator/Date	Verifier/Date
Aseptically inoculate the bioreactor per the process SOP.	Operator/Date	Verifier/Date
Immediately take a sample of the culture per the process SOP.	Operator/Date	Verifier/Date
Record all data obtained during sampling in the chart at the end of this batch record.	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

When glucose levels reach an undetectable level move to stage 2 of growth (methanol feed). Elapsed Time when moved to stage 2: _____hours OD when moved to stage 2: _____	Operator/Date	Verifier/Date
After 12-48 hours of methanol feed, harvest the culture.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
9. Data Collection and Cell Harvest		
Retrieve data generated by Biocommand Lite per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Using the sampling assembly, collect 1L of culture into sterile bottles through the harvest port.	Operator/Date	Verifier/Date
Transfer about 50mL of the culture into individual centrifuge tubes.	Operator/Date	Verifier/Date
Centrifuge at approximately 3000xg for 5-8 minutes. Remove supernatant and pour into sterile bottles by pouring into sterile bottles. Store at 2-8°C for use in Downstream Processing SOPs.	Operator/Date	Verifier/Date
Shut down and clean the BioFlo 3000 per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
10. Clean the BioFlo 3000		
Clean the BioFlo 3000 per the BioFlo 3000 SOP.	Operator/Date	Verifier/Date
11. Cryopreservation		
Autoclave 50mL of 100% glycerol in a 100mL bottle per autoclave SOP.	Operator/Date	Verifier/Date
In the BSC, sterilely transfer about 50mL of the culture into individual centrifuge tubes.	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

In the BSC pour off the supernatant into a waste container.	Operator/Date	Verifier/Date
Sterilely add 11mL of autoclaved glycerol to the 100mL of 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone set aside for cryopreservation in the process SOP to make the storage media.	Operator/Date	Verifier/Date
Aseptically add 5mL of the storage media to each centrifuge tube and resuspend the pelleted *Pichia* cells.	Operator/Date	Verifier/Date
Aseptically dispense 1mL aliquots to sterile 1.5mL cryovials. Label the cryovials: P. pastoris, HSA, [date], [initials], P[#]. Increase the passage number by one from the recorded Vial ID used in the seed flask culture.	Operator/Date	Verifier/Date
Place cryovials in a Styrofoam tube rack. Label container: P. pastoris, HSA, Working Cell Bank, [date], [initials], P[#]. Store at -86°C.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris* Upstream Process

HSA Lot Number_____

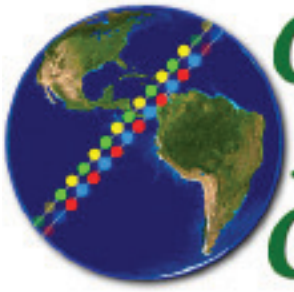
Elapsed Time (Hours)	pH	Temp (^{o}C)	%DO2	Agitation (rpm)	Methanol Feed	OD (600nm)	Glucose (mg/dL)	Operator/Verifier Date

Manufacturing Technician (Upstream)

REF	Key Functions & Tasks (Upstream Manufacturing Technician)
1	**Work in compliance with EH&S.**
1.a	Wear appropriate personal protective equipment.
1.b	Work in controlled environments.
1.c	Participate in emergency drills and emergency response teams.
1.d	Identify unsafe conditions and take corrective action.
1.e	Appropriately and safely access production equipment.
1.f	Handle, label, and dispose of hazardous / biohazard materials.
1.g	Access and utilize MSDS.
1.h	Perform permitting procedures.
1.i	Carries out operations with attention to OSHA and EPA regulations, and other applicable state and federal regulations.
1.j	Keeps work areas clean and safety equipment in order.
1.k	Participate in all company safety training and audits as required.
1.l	Assists with waste treatment operations.
2	**Work in compliance with cGMPs.**
2.a	Assists in environmental monitoring activities.
2.b	Follow SOPs for all operations.
2.c	Records process data and completes batch records as required.
2.d	Maintain equipment logbooks.
2.e	Control and receipt of raw materials.
2.f	Maintain training documentation.
2.g	Maintain equipment and processes in a validated state.
2.h	Working in controlled/classified areas (gowning, aseptic technique).
2.i	Ensure appropriate flow of personnel, equipment, and materials.
2.j	Change control for process, equipment, and documentation.
2.k	Label and apply status to equipment and materials.
2.l	Identify and report exception events and CAPA.
3	**Clean and maintain production areas.**
3.a	Housekeeping / pest control.
3.b	Sanitize and clean of controlled spaces.
3.c	Preparation of cleaning materials and solutions.
3.d	Assist in environmental monitoring for routine and changeover operations.
3.e	Document cleaning.
4	**Maintain effective communication.**
4.a	Deliver shift change update.
4.b	Communicate with coworkers and/or customers to ensure production or service meets requirements.
4.c	Suggest continuous improvements.
4.d	Coordinate with work teams / internal customers.
4.e	Maintain security and confidentiality.
4.f	Respond appropriately to internal auditors and external inspectors.
4.g	Assist in writing, reviewing, and commenting on technical documents.

REF	Key Functions & Tasks (Upstream Manufacturing Technician)
5	**Prepare process materials.**
5.a	Weigh, dispense, and label raw materials for use in production.
5.b	Dispense consumables and intermediates.
5.c	Control and reconcile inventory with enterprise control system (MRP, SAP, manual database).
5.d	Prepare and sterilize buffers and solutions.
5.e	Sample and test buffers and solutions.
5.f	Transfer buffers and solutions to use point.
5.g	Prepare filters for use.
5.h	Prepare, pasteurize / sterilize, and titrate media and feed solutions.
5.i	Manage chromatography resins.
6	**Prepare equipment.**
6.a	Clean CIP vessels, transfer lines, and filter trains .
6.b	Clean COP equipment (or sonicator).
6.c	Depyrogenate components and equipment.
6.d	Sterilize SIP vessels, transfer lines, and sampling ports.
6.e	Perform pressure test.
6.f	Prepare and assemble components and equipment.
6.g	Autoclave components and equipment.
6.h	Perform WFI flush of transfer lines.
6.i	Perform scheduled sanitizations of hoods.
6.j	Prepare and standardize probes and ancillary instruments.
6.k	Prepare, assembly, and integrity test filters.
6.l	Visually inspect equipment.
6.m	Maintain equipment logs and status tags.
6.n	Complete, review and approve equipment process records.
7	**Perform basic manufacturing operations.**
7.a	Perform processes following batch records, validation protocols, and/or SOPs.
7.b	Maintains and controls processes in an automated control environment.
7.c	Record process data.
7.d	Inspect materials at all stages of process to determine quality or condition.
7.e	Participate in the installation, modification, and upgrade of equipment.
7.f	Operate, monitor, and maintain equipment, tools, and workstation.
7.g	Recognize and respond appropriately to atypical events.
7.h	Participate in troubleshooting and root cause analysis of operations.
8	**Perform upstream manufacturing operations.**
8.a	Work in an aseptic environment (laminar flow hood / biosafety cabinet / cleanrooms).
8.b	Perform vial thaw from a working cell bank.
8.c	Perform cell culture expansion.
8.d	Monitor cell concentration by cell counting or measuring OD.
8.e	Inoculate seed reactor.
8.f	Transfer of seed culture to production reactors.
8.g	Monitor and control growth of cells in batch, fed-batch, and perfusion reactors.
8.h	Perform aseptic additions of media, solutions, and/or gases to reactors.
8.i	Perform CIP/SIP of bioreactors.
9	**Perform Sampling.**
9.a	Prepare sample port for aseptic sampling.
9.b	Obtain in-process samples according to batch records or sampling plans.
9.c	Label samples appropriately.
9.d	Record sample collection and distribution (storage and chain of custody).
9.e	Perform in-process chemical and/or microbiological tests.

Downstream



GLOBAL
BIOMANUFACTURING
CURRICULUM

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Title: Ion Exchange Chromatography of tPA SOP

Approvals:
Preparer:_____Kari Britt_____ Date ____02Jun09_____
Reviewer:_____Bob O'Brien_____ Date ____02Jun09_____

1. **Purpose:**
   1.1. To purify tPA using ion exchange chromatography.
2. **Scope:**
   2.1. Applies to purifying tPA using POROS 50HS resin and the BioLogic LP system.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. POROS 50HS Manufacturer's Instructions.
   4.2. pH meter SOP
   4.3. Amicon/Millipore column assembly SOP
   4.4. BioLogic LP SOP
5. **Definitions:**
   5.1. CV: Column Volume; CV = π(L in cm)[(radius of column in cm)^2]
   5.2. L = Length of column (meaning the height of the bead bed)
   5.3. HETP: Height Equivalent to Theoretical Plate; HETP = L/N
   5.4. $N = 5.54\ (t_R/w_{1/2})^2$
   5.5. t_R: retention time
   5.6. $w_{1/2}$: peak width at half height
   5.7. h: Reduced Plate Height; h = HETP/Dp
   5.8. Dp: bead diameter
6. **Precautions:**
   6.1. 0.1M NaOH is very corrosive. It is extremely damaging to eyes and mucous membranes. It causes burns. Avoid contact with skin. Harmful if swallowed or inhaled.
7. **Materials:**
   7.1. Amicon Vantage-L Biochromatography column and accessories
   7.2. POROS 50 HS Cation exchange packing medium (2-8°C)
   7.3. BioRad BioLogic LP System
   7.4. 0.22 µm sterile filter units
   7.5. pH paper
   7.6. waste beakers
   7.7. laboratory film, such as, Parafilm
   7.8. ring stand with clamps
   7.9. 1mL syringe
   7.10. MilliQ water
   7.11. Equilibration Buffer A: 20mM phosphate buffer pH 6
      7.11.1. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)

Title: Ion Exchange Chromatography of tPA SOP

7.11.2. Na_2HPO_4-$7H_2O$ (sodium phosphate dibasic, heptahydrate)
7.12. Elution Buffer B: 20mM phosphate buffer pH6, 1M NaCl
7.12.1. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)
7.12.2. Na_2HPO_4-$7H_2O$ (sodium phosphate dibasic, heptahydrate)
7.12.3. NaCl (sodium chloride)
7.13. Cleaning Solution 0.1M NaOH

8. Procedure:

8.1. **Prepare buffers and solutions**

8.1.1. **Buffer A: Equilibration Buffer, 20mM Phosphate, pH 6**

8.1.1.1. Weigh 2.10 ±0.05 grams of sodium phosphate monobasic anhydrous.
8.1.1.2. Weigh 0.66 ±0.02 grams of sodium phosphate dibasic heptahydrate.
8.1.1.3. Transfer both chemicals to a 1L vessel.
8.1.1.4. Using a 1L graduated cylinder, measure approximately 1L of deionized water.
8.1.1.5. Transfer water to the 1L vessel.
8.1.1.6. Add magnetic stir bar and stir to dissolve.
8.1.1.7. Adjust pH to 6 ±0.1.
8.1.1.8. Filter sterilize.
8.1.1.9. Label as: Buffer A, Equilibration Buffer, 20mM Phosphate, pH 6, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.2. **Buffer B: Elution Buffer, 20mM phosphate, pH 6, 1M NaCl**

8.1.2.1. Weigh 29.2 ±0.2 grams NaCl.
8.1.2.2. Transfer to a 1L vessel.
8.1.2.3. Using a 500mL graduated cylinder, measure approximately 500mL of 20mM phosphate buffer pH 6.
8.1.2.4. Transfer to the 1L vessel with the NaCl.
8.1.2.5. Add magnetic stir bar and stir to dissolve.
8.1.2.6. Filter sterilize.
8.1.2.7. Label as: Buffer B, Elution Buffer, 20mM Phosphate, pH 6, 1M NaCl, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.3. **Cleaning Solution: 0.1M NaOH**

8.1.3.1. Weigh 2.0 ±0.05 grams of NaOH.
8.1.3.2. Transfer NaOH to a 1L vessel.
8.1.3.3. Using a 500mL graduated cylinder, measure 500mL of deionized water.
8.1.3.4. Transfer to the 1L vessel.
8.1.3.5. Add magnetic stir bar and stir to dissolve.
8.1.3.6. Filter sterilize.
8.1.3.7. Label as: Cleaning Solution, 0.1M NaOH, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.4. **Buffer C: CHO Cell Culture Supernatant, pH 6**

8.1.4.1. Adjust the pH of the CHO cell culture supernatant obtained during upstream processing of tPA to pH 6.
8.1.4.1.1. Measure the pH of the CHO cell culture supernatant per pH meter SOP.

Title: Ion Exchange Chromatography of tPA SOP

8.1.4.1.2. If the initial pH is above 6 ±0.1, carefully add one drop of hydrochloric acid solution to the supernatant.
8.1.4.1.3. Observe the change in pH.
8.1.4.1.4. Repeat step 8.1.4.1. until the supernatant solution is pH 6 ±0.1.
8.1.4.2. Label as: Buffer C, CHO Cell Culture Supernatant, pH 6, Store: 2-8°C, Dispose: drain, [date], [group], [initials].

8.2. **Purge BioLogic LP Sytem with Buffer A and zero the UV monitor per the Biologic LP Chromatography System SOP**

8.3. **Pour Column per the BioLogic LP Chromatography System SOP**

8.3.1. Use approximately 5mL of POROS HS resin.

8.4. **Attach the column to the BioLogic LP per the BioLogic LP Chromatography System SOP**

8.5. **Pack Column per the BioLogic LP Chromatography System SOP**

8.5.1. Place the line for Buffer A into the vessel containing Buffer A, Equilibration Buffer. Cover the opening of the vessel with laboratory film, such as Parafilm.

8.5.2. Use Method: IEX Pack:

Step 1:	0-5min	Buffer A	1.0mL/min
Step 2:	5-10min	Buffer A	2.0mL/min
Step 3:	10-20min	Buffer A	3.0mL/min
Step 4:	25-30min	Buffer A	6.0mL/min

8.6. **Determine the HETP and h of the column per the BioLogic LP Chromatography System SOP.**

8.6.1. Use Method: IEX HETP

Step1:	0-15min	Buffer A	5mL/min

8.6.2. The Dp of the bead is 0.05mm.

8.6.3. The expected HETP is approximately 0.1mm.

8.6.4. The h calculation should be less than 3. If h is greater than 3, the desired product may not bind the column efficiently. In this case it is best to re-pack the column.

8.7. **Run Column per the BioLogic LP Chromatography System SOP.**

8.7.1. Place the lines for Buffers A, B and C into the vessels containing the appropriate buffers. Cover the opening of each vessel with laboratory film.

8.7.2. Use Method: IEX tPA

Step 1:	0 to 20min	Buffer C	4mL/min
Step 2:	20 to 40min	Buffer A	4mL/min
Step 3:	40 to 60min	Buffer B	4mL/min
Step 4:	60 to 80min	Buffer A	4mL/min

8.7.3. Collect 1-5mL of the flow through fraction when the first A.U. peak begins to plateau (approximately 5 minutes into the run).

8.7.4. Collect the entire elution fraction when the second A.U. peak BEGINS to appear (approximately 40 minutes into the run).

8.7.5. Store fractions at 2-8^{o}C for SDS PAGE analysis.

8.8. **Clean the Column per the BioLogic LP Chromatography System SOP.**

8.8.1. Place the lines for buffers A and B into the vessel containing Cleaning Solution, 0.1M NaOH. Cover the opening of the vessel with laboratory film.

Title: Ion Exchange Chromatography of tPA SOP

8.8.2. Use Method: IEX Clean

Step 1: 0 to 20min Buffer 50% B 4mL/min

8.9. **Store the System per the BioLogic LP Chromatography System SOP.**

9. Attachments: N/A

10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
Deb Audino	7/2005	Changed from manual pump system to BioLogic LP system.
Deb Audino	051206	Removed steps associated with equipment operation to simplify the process SOP.
Deb Audino	31Aug07	Simplified the packing method.
Deb Audino	04Apr08	College name change
Kari Britt	31May09	Added labeling directions, directions for covering vessels with laboratory film, and directions for placing buffer lines into the appropriate buffer. Also made general grammar and formatting edits as needed throughout the document.

Title: BioLogic LP Chromatography System Operating SOP

Approvals:
Preparer: ___Kari Britt _____ Date:_____02Aug10_____
Reviewer: __Sonia Wallman_____ Date:_____02Aug10_____

1. **Purpose:** Operation of the BioLogic LP Chromatography System.
2. **Scope:** Applies to the BioLogic LP Chromatography System for purifying proteins.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. BioLogic LP Chromatography System Instruction Manual
5. **Definitions**:
   5.1. CV: Column Volume; $CV = \pi(L \text{ in cm})[(\text{radius of column in cm})^2]$
   5.2. L = Length of column (meaning the height of the bead bed)
   5.3. HETP: Height Equivalent to Theoretical Plate; HETP = L/N
   5.4. $N = 5.54 (t_R/w_{1/2})^2$
   5.5. t_R: retention time
   5.6. $w_{1/2}$: peak width at half height
   5.7. h: Reduced Plate Height; h = HETP/Dp
   5.8. Dp: bead diameter
6. **Precautions**: N/A
7. **Materials:**
   7.1. deionized Water
   7.2. Equilibration Buffer A (Refer to the process SOP)
   7.3. Equilibration Buffer B (Refer to the process SOP)
   7.4. Cleaning Solution (Refer to the process SOP)
   7.5. biopure water
   7.6. container for waste fluid
   7.7. collection tubes for fraction collector or collection containers
   7.8. column (Amicon Vantage-L Biochromatography column and accessories)
   7.9. resin (Refer to the process SOP.)
   7.10. lab towels
8. **Procedure:**
   8.1. Turn on BioLogic LP system (switch is in the front, on the lower left side of the system).
   8.2. Turn on computer.
   8.3. Click on the LP DataView icon.
   8.4. Verify that the computer is communicating with the system as indicated by a green "Receive" circle on the upper right side of the computer screen.
   8.5. **Pump Calibration**

Title: BioLogic LP Chromatography System Operating SOP

8.5.1. Based on the desired flow rate, select the appropriate tubing for the pump as follows:
Flow rates of 0.04-0.8 mL/min require 0.8mm tubing.
Flow rates of 0.2-4.0 mL/min require 1.6mm tubing.
Flow rates of 0.8-15.0 mL/min require 3.2mm tubing.

8.5.2. Verify that the correct tubing is in the pump.

8.5.2.1. Remove the platen by lifting the grey handle (Figure 2).

8.5.2.2. If necessary, insert the correct tubing.

8.5.2.3. Replace platen and lock into place.

8.5.2.4. If tubing was replaced readjust the platen and recalibrate the pump.

8.5.2.4.1. Loosen the platen adjust screw located on the top of the pump (Figure 2) by turning counterclockwise until there is slight resistance.

8.5.2.4.2. Tighten the platen screw clockwise the appropriate number of COMPLETE turns.
0.8mm tubing requires 5 turns
1.6mm tubing requires 4 turns
3.2mm tubing requires 3 turns

8.5.2.5. Recalibrate the pump.

8.5.2.5.1. Press the MANUAL mode key.

8.5.2.5.2. Press the PUMP instrument key.

8.5.2.5.3. Select FLOW, then select CALIBRATE.

8.5.2.5.4. Select the appropriate tubing size.

8.5.2.5.5. Select NOMINAL.

8.6. **Purge System with Buffer A and Zero the UV Monitor**

8.6.1. Place each buffer line into a container filled with Buffer A (Equilibration Buffer).

8.6.2. Attach the column inlet tube directly to the column outlet tube.

8.6.3. Press the MANUAL mode key.

8.6.4. Select BUFFER.

8.6.5. Select MIX.

8.6.6. Type in 50% B.

8.6.7. Select OK.

8.6.8. Select PURGE.

8.6.9. Allow system to purge until conductivity reading on the display panel of the Biologic LP system controller stabilizes (less than 5 minutes).

8.6.10. Select BUFFER.

8.6.11. Using the arrow key, select C.

8.6.12. Select OK.

8.6.13. Allow system to purge until conductivity reading on the display panel of the controller stabilizes (less than 5 minutes).

8.6.13.1. While the system is running, zero the UV Monitor.

8.6.13.1.1. Press the UV instrument key.

8.6.13.1.2. Select ZERO.

Title: BioLogic LP Chromatography System Operating SOP

8.6.13.1.3. Verify that the absorbance changes to zero on the display panel of the controller.
8.6.13.1.4. Press the PUMP instrument key.
8.6.14. After conductivity stabilizes, select STOP.

8.7. **Pour the Column (if necessary)**
8.7.1. Secure the column in an upright position to a stand using clamps.
8.7.2. Close the bottom valve on the column (handle should be in the horizontal position).
8.7.3. Add approximately 10mL Buffer A to the column.
8.7.4. Obtain the appropriate resin (Refer to the process SOP.).
8.7.5. Swirl the resin to make a homogeneous mixture.
8.7.6. Measure the appropriate amount of resin (Refer to the process SOP.) with a graduated cylinder and transfer to the column.
8.7.7. Dislodge any beads that stick to the column with additional buffer.
8.7.8. Position the 3 way valve on the top adapter to close the top port (handle points to the top port).
8.7.9. Place the tubing from 3 way valve into the waste container.
8.7.10. Secure the adapter housing to the glass column.
8.7.11. Allow the resin to settle until there is a clear layer of buffer above the surface of the resin.
8.7.12. Depress the top adapter until it reached approximately 3cm above the resin, making sure air and then liquid comes out the top of the 3 way valve.
8.7.13. Lock the adapter into place.

8.8. **Attach the Column**
8.8.1. Position the 3 way valve to close off the column (handle points to the column).
8.8.2. Disconnect the column inlet and outlet tubing from the tubing connector.
8.8.3. Attach the column inlet tubing from the injector valve to the top of the column 3 way valve.
8.8.4. Attach the column outlet tubing to the bottom of the column.
8.8.5. Place tubing from the 3 way valve side port in the waste container.
8.8.6. Open the valve at the bottom of the column (handle in vertical position).
8.8.7. Press MANUAL mode key.
8.8.8. Select PURGE.
8.8.9. Allow buffer to drip into the waste container from the side port until air bubbles are completely absent from the tubing.
8.8.10. Simultaneously select STOP and position the 3 way valve to close the side port.

8.9. **Pack the Column**
8.9.1. Place all lines in the appropriate buffers/solutions as per the process SOP.
8.9.2. Press the PROGRAM mode key.
8.9.3. Select LIST METHODS.

Title: BioLogic LP Chromatography System Operating SOP

8.9.4. Using the arrow keys, select the correct method (Refer to the process SOP.). If the method is not listed, refer to the Biologic LP Chromatography System Instruction Manual to create a new program.
8.9.5. Select OPEN.
8.9.6. Using the arrow keys, verify that the method agrees with the process SOP.
8.9.6.1. If the method has been changed, refer to the Biologic LP Chromatography System Instruction Manual to edit the program.
8.9.7. Select DONE.
8.9.8. Press the RUN mode key.
8.9.9. System will have a 10 second delay.
8.9.10. Verify that the computer is recording data by the appearance of an S symbol on the graph.
8.9.10.1. If the S is not present, click the "record" button on the toolbar on the computer screen.
8.9.11. Once the method is finished, unlock the top adapter, lower the top adapter down to ~2mm above the beds and then re-lock the adapter.
8.9.11.1. Measure the bed height.
8.9.11.2. Determine the Column Volume for your column using the following formula: $CV = \pi(L \text{ in cm})[(\text{radius of column in cm})^2]$
Refer to Definitions (section 5) as needed to complete the calculation.
8.9.11.3. There is no need to save the chromatogram, clear the screen using the "clear" button on the toolbar.

8.10. **Determine the HETP and h**
8.10.1. Attach an appropriate size sample loop to the sample valve (usually a 125µL loop is appropriate).
8.10.2. Turn MV-6 injector valve knob counterclockwise until there is resistance.
8.10.3. Draw 1mL of elution buffer into a syringe.
Note: If the elution buffer does not contain salt, then sterile filter a 1M sodium chloride solution to inject into the system.
8.10.4. Insert syringe into top port. Push slowly to fill sample loop while simultaneously collecting overflow in a beaker.
8.10.5. Leave syringe in port.
8.10.6. Turn MV-6 injector valve knob clockwise until there is resistance.
8.10.7. Press the PROGRAM mode key.
8.10.8. Select LIST METHODS.
8.10.9. Using the arrow keys, select the correct method (refer to the process SOP). If the method is not listed, refer to the Biologic LP Chromatography System Instruction Manual to create a new program.
8.10.10. Select OPEN.
8.10.11. Using the arrow keys, verify that the method agrees with the process SOP.
8.10.11.1. If the method has been changed, refer to the Biologic LP Chromatography System Instruction manual to edit the program.
8.10.12. Select DONE.

Title: BioLogic LP Chromatography System Operating SOP

8.10.13. Press the RUN mode key.
8.10.14. System will have a 10 second delay.
8.10.15. Verify that the computer is recording data by the appearance of an S symbol on the graph.
8.10.15.1. If the S symbol is not present, click the "record" button on the toolbar on the computer screen.
8.10.16. Once a full peak has been generated, stop the program.
8.10.17. Turn MV-6 injector valve knob counterclockwise until there is resistance.
8.10.18. Save and print the file, making note of the directory where the chromatogram was saved.
8.10.19. Clear the screen.
8.10.20. From the chromatogram determine the HETP and h.

HETP = L/N

Note: Use L in mm for this calculation.

$N = 5.54 (t_R/w_{1/2})^2$

h = HETP/Dp

Refer to Definitions (section 5) and Figure 5 as needed to complete the calculations. Refer to the process SOP for the Dp value.

8.11. **Run the Column**
8.11.1. Place all lines in the appropriate buffers/solutions as per the process SOP.
8.11.2. Press the PROGRAM mode key.
8.11.3. Select LIST METHODS.
8.11.4. Using the arrow keys, select the correct method as per the process SOP. If the method is not listed, refer to the Biologic LP Chromatography System Instruction Manual to create a new program.
8.11.5. Select OPEN.
8.11.6. Using the arrow keys, verify that the method has not been changed.
8.11.6.1. If the method has been changed, refer to the Biologic LP Chromatography System Instruction Manual to edit the program.
8.11.7. Select DONE.
8.11.8. Press the "Run" mode key.
8.11.9. System will have a 10 second delay.
8.11.10. Verify that the computer is recording data by the appearance of an S symbol on the graph.
8.11.10.1. If the S symbol is not present, click the "record" button on the toolbar on the computer screen.

8.12. **Clean the Column**
8.12.1. Place buffer lines into the appropriate cleaning solution (Refer to the process SOP.).
8.12.2. Run the appropriate cleaning method (Refer to the process SOP.).

8.13. **Clean and Store the System**
8.13.1. If the system will be used again with the same column within a few days, it may be stored "as is" after a run.

Title: BioLogic LP Chromatography System Operating SOP

8.13.1.1. Turn off the system and turn off the computer.

8.13.2. If the system will not be used within a few days it must be flushed with water then 20% ethanol and purged with air.

8.13.2.1. Disconnect the column.
8.13.2.2. Attach the column inlet tube directly to the column outlet tube.
8.13.2.3. Place each buffer line into a container filled with biopure water.
8.13.2.4. Attach the column inlet tube directly to the column outlet tube.
8.13.2.5. Press the MANUAL mode key.
8.13.2.6. Select BUFFER, then select MIX.
8.13.2.7. Type in 50% B, then select OK.
8.13.2.8. Select PURGE.
8.13.2.9. Allow system to purge until conductivity reading stabilizes (less than 5 minutes).
8.13.2.10. Select BUFFER.
8.13.2.11. Using the arrow key, select C.
8.13.2.12. Select OK.
8.13.2.13. Allow system to purge until conductivity reading stabilizes (less than 5 minutes).
8.13.2.14. Select STOP.
8.13.2.15. Place each buffer line into 20% Ethanol and repeat steps 8.13.2.6. through 8.13.2.16.
8.13.2.16. Place each buffer line on a lab towel or kimwipes so that they are open to the air and repeat steps 8.13.2.6. through 8.13.2.16.
8.13.2.17. Turn off the LP Biologic System.

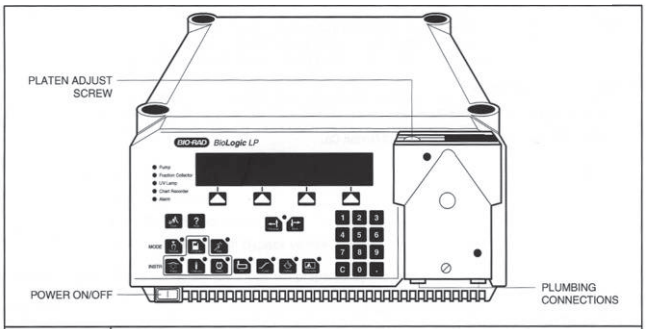
9. Attachments:

9.1. Figure 1: Controller Front Panel
9.2. Figure 2: Controller Pump
9.3. Figure 3: LP Biologic System Parts
9.4. Figure 4: Column components
9.5. Figure 5: Chromatogram example for calculating HETP

10. History:

Name	Date	Amendment
Deb Audino	070105	Initial release
Deb Audino	110405	Removed purging the system with water and Buffer B prior to use. Added the cleaning and storing section.
Deb Audino	17May06	Added the column components figure, added steps that were removed from the process SOPs.
Bob O'Brien	23Jan08	Added steps to clarify use of the 3 way valve.
Deb Audino	04Apr08	College name change
Kari Britt	03Aug10	Added to definitions and HETP sections. Added Figure 5. Made grammar and formatting edits as needed throughout the document. Removed references to programming SOP.

Title: BioLogic LP Chromatography System Operating SOP



Feature	Description
Control Panel	Consists of the control keys and status LEDs for monitoring and controlling the system. It is designed to withstand the minor spills associated with use in a laboratory.
Power switch	Turns on/off the BioLogic LP Controller.
Plumbing Connections	The peristaltic pump may be used with most flexible tubing having an inner diameter less than or equal to 3.2 mm (1/8") and a wall thickness of 1.0 mm or less, including PharMed, and silicone. Inlet and outlet lines attach to the ports at the bottom of the pump. These ports accept standard luer fittings.

Figure 1: Controller Front Panel

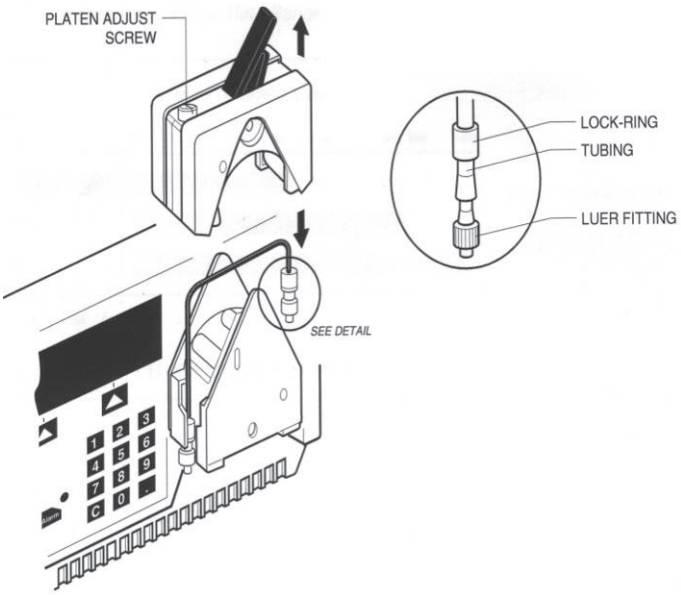


Figure 2: Controller Pump

Title: BioLogic LP Chromatography System Operating SOP

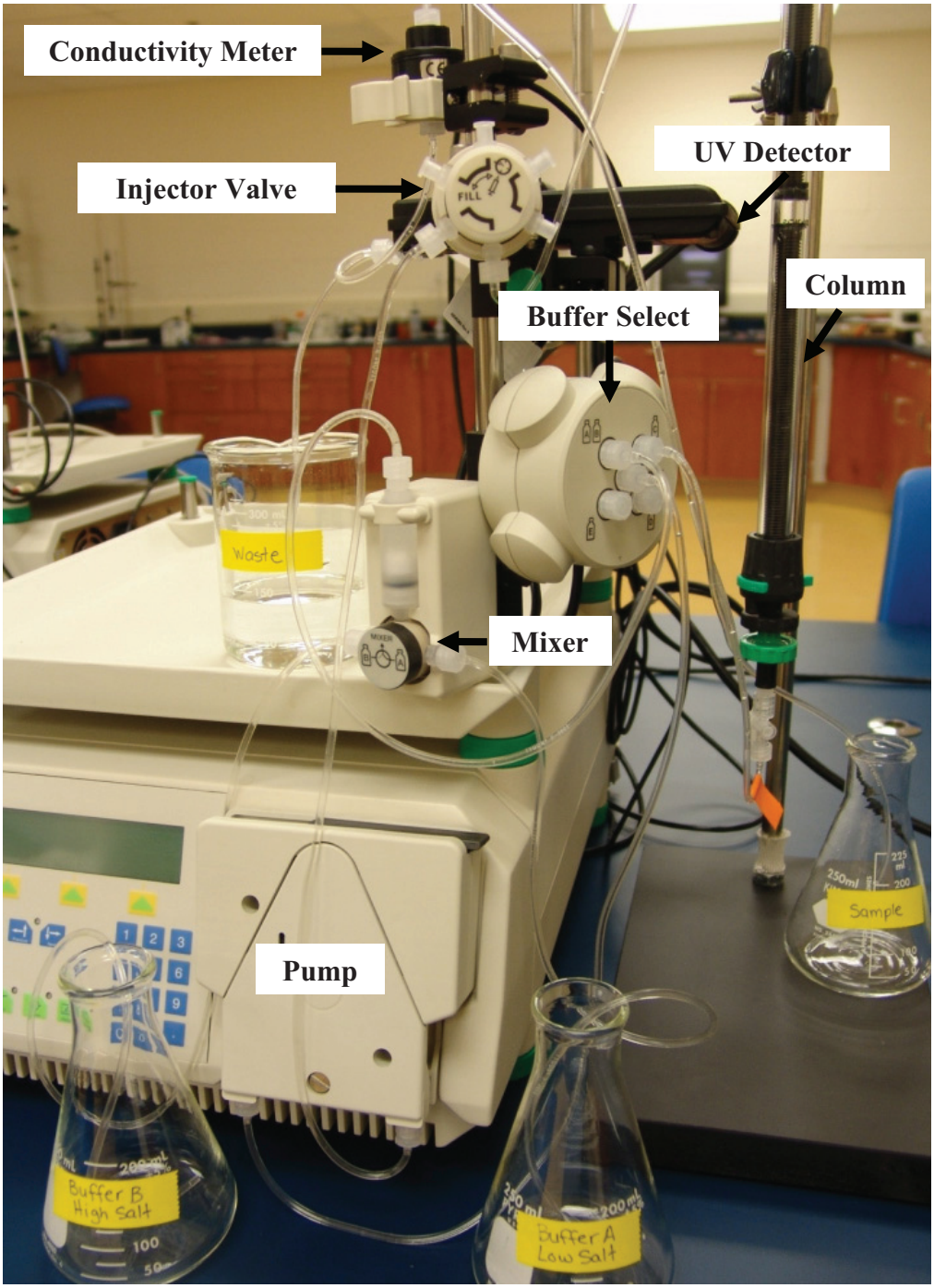


Figure 3: LP Biologic System Parts

Title: BioLogic LP Chromatography System Operating SOP

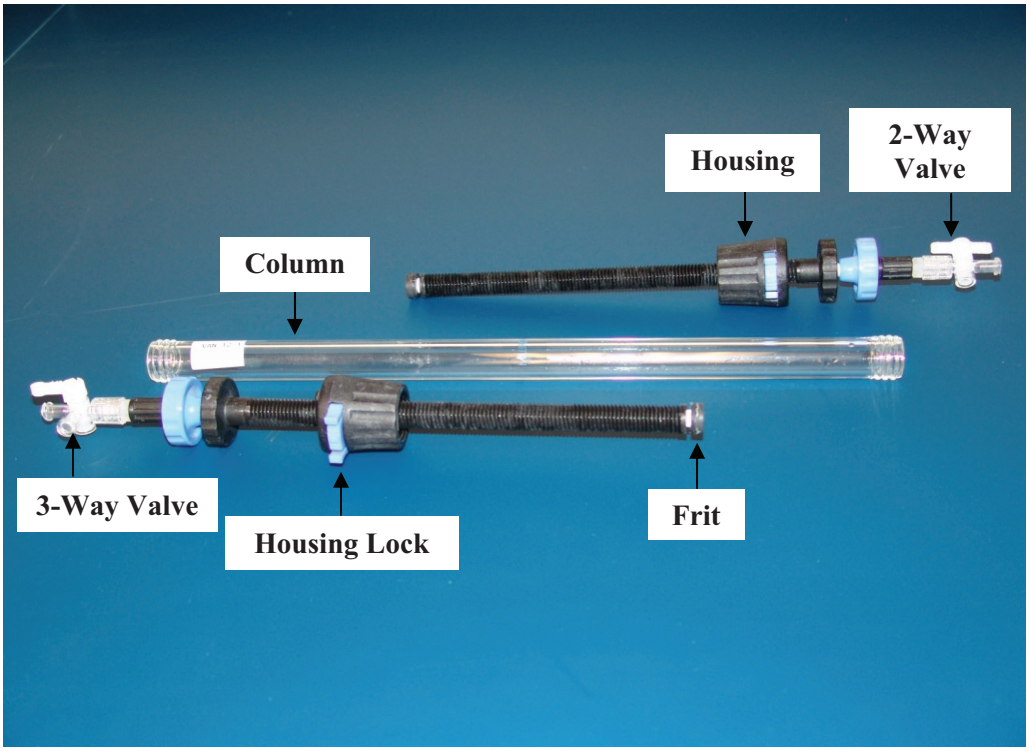


Figure 4: Column Components

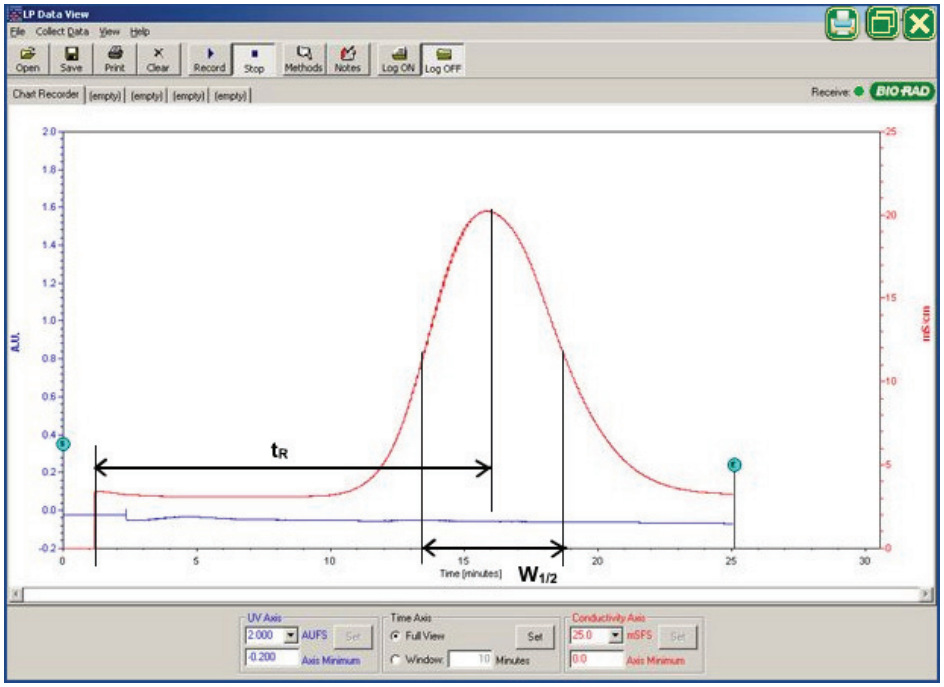


Figure 5: Chromatogram Example for Calculating HETP

Batch Record: tPA Production from CHO Cells Downstream Process
tPA Lot Number_____

Record Keeping Standards:

For each step in the batch record: the operator of the task will enter their initials (each operator has their own unique set of initials) and the date in the appropriate section(s) of the batch record. Another operator must initial and date in the appropriate section of the batch record to verify that the task was completed per SOP. No operator will verify their own work at any point. "If you didn't document it, you didn't do it!"

Batch records will be completed in blue or black ball point pen ONLY, and must be legible.

Any errors on a batch record will be crossed out with a single line through the error with the initials of the operator and the date. Corrections will be written in next to the crossed out error.

Use the following format to record dates: DDMMMYY. For July 10, 2006 use 10JUL06.

Use the 24 hour clock or "military time" to record time: 3:00pm would be written as 15:00.

Any and all deviations from a protocol or SOP, including abnormal results or retests performed, will be entered into the comments section at the end of each batch record. Be as detailed and specific as possible, include all steps taken before and/or after an abnormal reading, and provide an explanation for any deviations from a step.

Batch Record: tPA Production from CHO Cells Downstream Process
tPA Lot Number_____

1. Solution and Buffer Preparation Buffer A: Equilibration Buffer, 20mM phosphate, pH 6. Buffer B: Elution Buffer, 20mM phosphate pH 6, 1M NaCl Cleaning Solution: 0.1M NaOH Adjust cell culture supernatant to pH 6.		
Calibrate pH meter per SOP with commercially prepared standard buffers (pH 7 and pH 4): pH Meter ID #_____ pH 7 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ pH 4 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Weigh 2.10 ±0.05 grams of sodium phosphate monobasic anhydrous. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Weigh 0.66 ±0.02 grams of sodium phosphate dibasic heptahydrate. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve sodium phosphate monobasic anhydrous with the sodium phosphate dibasic heptahydrate in approximately 1L of deionized water using magnetic stir bar. Volume of water added _____ mL	Operator/Date	Verifier/Date
Adjust Equilibration Buffer A to pH 6.0 ±0.1. pH_____	Operator/Date	Verifier/Date
Sterile filter solution and label as: Buffer A, Equilibration Buffer, 20mM Phosphate, pH 6, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Downstream Process
tPA Lot Number_____

Weigh 29.2 ±0.2 grams NaCl. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve in approximately 500ml of Equilibration Buffer A using magnetic stir bar. Volume of Buffer A added _____ mL	Operator/Date	Verifier/Date
Sterile filter solution and label as: Buffer B, Elution Buffer, 20mM Phosphate, pH 6, 1M NaCl, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Weigh 2.0 ±0.05 grams of NaOH. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve in approximately 500ml deionized water using magnetic stir bar. Volume of water added _____ mL	Operator/Date	Verifier/Date
Sterile filter solution and label as Cleaning Solution, 0.1M NaOH, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Adjust pH of CHO cell culture supernatant to pH 6 ±0.1. pH_____	Operator/Date	Verifier/Date
Label CHO cell culture supernatant as: Buffer C, CHO Cell Culture Supernatant, pH 6, Store: 2-8°C, Dispose: drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Downstream Process
tPA Lot Number_____

2. Purge BioLogic LP System, Pour Column and Attach to Biologic LP System		
Calibrate pump if necessary per the BioLogic LP Chromatography System SOP. **Verify** that **1.6mm** tubing is in the pump. Change tubing if necessary. Tubing changed: Yes / No (Circle one.) If the tubing was changed, adjust the platen and calibrate the pump per BioLogic LP SOP. Platen adjusted: Yes / No (Circle one.) Pump recalibrated: Yes / No (Circle one.)	Operator/Date	Verifier/Date
Purge the BioLogic LP system with Buffer A per the Biologic LP Chromatography System SOP.	Operator/Date	Verifier/Date
Place each buffer line into a container filled with Buffer A (Equilibration Buffer).	Operator/Date	Verifier/Date
Zero the UV monitor per the Biologic LP Chromatography System SOP.	Operator/Date	Verifier/Date
Add approximately 5ml of POROS 50 HS resin to column per BioLogic LP Chromatography System SOP. Volume of POROS 50 HS added: _____ mL Amicon Vantage-L-Column ID#:_____	Operator/Date	Verifier/Date
Attach the column to the BioLogic LP per the BioLogic LP Chromatography System SOP. BioLogic LP ID#:_____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Downstream Process
tPA Lot Number_____

3. Pack the Column and Determine HETP and h		
Pack column per the BioLogic LP Chromatography System SOP using Method: IEX Pack.	Operator/Date	Verifier/Date
Place the line for Buffer A into the vessel containing Buffer A, Equilibration Buffer. Cover the opening of the vessel with laboratory film, such as Parafilm.	Operator/Date	Verifier/Date
Determine column volume per the BioLogic LP Chromatography System SOP. CV = π(bed height in cm)(radius of column in cm)^2 Write out CV calculation in this space: Bed Height: _____ Column Volume: _____	Operator/Date	Verifier/Date
Produce chromatogram needed to determine HETP and h per BioLogic LP Chromatography System SOP using Method: IEX HETP. Volume of 0.8M NaCl loaded_____mL	Operator/Date	Verifier/Date
Determine HETP of the column per BioLogic LP Chromatography System SOP and **attach** chromatogram to batch record. Dp = 0.05mm for POROS HS resin. Write out HETP and h calculations in this space: HETP value:_____mm h value:_____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Downstream Process
tPA Lot Number_____

4. Run Column		
Run column per the BioLogic LP Chromatography System SOP using Method: IEX tPA.	Operator/Date	Verifier/Date
Place the lines for Buffers A, B and C into the vessels containing the appropriate buffers. Cover the opening of each vessel with laboratory film.	Operator/Date	Verifier/Date
Store fractions at 2 – 8ºC for SDS PAGE Analysis.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
5. Clean and Store BioLogic LP Chromatography System		
Clean the column per the BioLogic LP Chromatography System SOP using Method: IEX Clean.	Operator/Date	Verifier/Date
Place the lines for buffers A and B into the vessel containing Cleaning Solution, 0.1M NaOH. Cover the opening of the vessel with laboratory film.	Operator/Date	Verifier/Date
Clean and store the BioLogic LP Chromatography System per the BioLogic LP Chromatography System SOP. Column Storage (Check one): Left on Biologic System _____ Disconnected and stored at room temp. _____ Disconnected and stored at 2-8C _____ Disassembled _____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Title: Tangential Flow and Diafiltration of HSA

Approvals:
Preparer:_____Deb Audino _____ Date ____03Apr08_____
Reviewer:_____Bob O'Brien_____ Date____ 03Apr08_____

1. **Purpose:**
   1.1. To concentrate and perform buffer exchange of protein product using tangential flow and diafiltration processes.
2. **Scope:**
   2.1. Applies to the concentration and buffer exchange of HSA from *Pichia pastoris*.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. pH meter SOP
   4.2. Millipore Pellicon XL Tangential Apparatus SOP
5. **Definitions:**
   5.1. Permeate- the material that passes through the membrane
   5.2. Retentate- the material that does not pass through the membrane
6. **Precautions:** N/A
7. **Materials:**
   7.1. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)
   7.2. Na_2HPO_4-$7H_2O$ (sodium phosphate dibasic, heptahydrate)
   7.3. 1L container
   7.4. 1L graduated cylinder
   7.5. 1L filter unit
   7.6. pH Meter and pH paper
   7.7. magnetic stir plate and stir bars
   7.8. Millipore Pellicon XL Tangential Apparatus (BioMax 50 Regenerated Cellulose MWCO 10) and accessories.
8. **Procedure:**
   8.1. Diafiltration Buffer Preparation (20mM Phosphate Buffer pH 7.1)
      8.1.1. Weigh out 0.80±0.02g NaH_2PO_4 and place into a 1L container.
      8.1.2. Weigh out 3.60±0.2g of Na_2HPO_4-$7H_2O$ and place into the 1L container with the NaH_2PO_4.
      8.1.3. Using a 1L graduated cylinder, measure 1L of deionized water.
      8.1.4. Transfer water to the 1L flask.
      8.1.5. Add magnetic stir bar and stir to dissolve.
      8.1.6. Adjust pH to 7.1±0.1.
      8.1.7. Sterile Filter the solution and label container: 20mM Phosphate Buffer pH 7.1, [date], [initials], [group], storage: room temp, disposal: drain.
   8.2. Set up the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.

Title: Tangential Flow and Diafiltration of HSA

8.3. Flush the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.
8.4. Precondition the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.
8.5. **Concentrate the sample.**
  8.5.1. Fill the feed container with sample to be concentrated.
  8.5.2. Place the ends of the retentate and feed tubes into the feed container and place the permeate tube into a separate container.
  8.5.3. Add stir bar to the sample and place on a stir plate. Slowly stir the sample.
  8.5.4. With thumbscrew still lightly tightened, turn on the pump, which has been set at a flow rate of 30-50 mL/min.
  8.5.5. Filter the solution until the desired volume is reduced 10-fold.
  8.5.6. Turn off the pump and empty the permeate container into a large bottle with cap. Label the bottle: HSA Permeate Waste, disposal: bleach then drain, [initials], [date].
8.6. **Perform a buffer exchange on the sample**.
  8.6.1. Add the 20mM Phosphate Buffer to the sample to bring the volume back to the pre-concentrated volume.
  8.6.2. Repeat step 8.5 until the pH of the concentrated retentate is 7.1±0.1 as measured with a pH meter.
8.7. **Retrieve the Sample**
  8.7.1. Turn the pump off and remove the feed tube from the feed container.
  8.7.2. Turn the pump on and pump all the retentate into the feed container.
  8.7.3. Turn the pump off.
  8.7.4. Remove the concentrated sample. Label as: Concentrated HSA, [date], [initials].
  8.7.5. Store in 2°C – 8°C refrigerator until column chromatography step.
8.8. Flush the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.
8.9. Clean the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.
8.10. Store the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.

9. Attachments: N/A

10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
SCP	2003	
Deb Audino	04Nov05	Put into 2005 SOP format. Separated out tPA and HSA procedures. Simplified the preparation of the 20mM phosphate buffer step
Deb Audino	04Apr08	college name change

Title: Millipore Pellicon XL Tangential Flow Filter SOP

Approvals:
Preparer:_____Deb Audino_____ Date _____03Apr08_____
Reviewer:_____Bob O'Brien _____ Date _____03Apr08_____

1. **Purpose:**
   1.1. To perform tangential flow filtration.
2. **Scope:**
   2.1. Applies to performing Tangential Flow Filtration with the Millipore Pellicon XL Tangential Apparatus to concentrate and perform buffer exchange.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Millipore Pellicon XL Operations Manual
   4.2. pH meter SOP
5. **Definitions:**
   5.1. Permeate- the material that passes through the membrane.
   5.2. Retentate- the material that does not pass through the membrane.
6. **Precautions:**
   6.1. 0.1M NaOH is very corrosive. It is extremely damaging to eyes and mucous membranes. It causes burns. Avoid contact with skin. It is harmful if swallowed or inhaled. The Millipore Pellicon XL Tangential Apparatus is stored and flushed with 0.1M NaOH.
   6.2. NEVER tighten the clamp enough to completely restrict the flow in the Retentate tube. This could damage the filter and cause the tubing to disconnect.
7. **Materials:**
   7.1. 0.1M NaOH (sodium hydroxide)
   7.2. preconditioning buffer
   7.3. pH Meter and pH paper
   7.4. 1L filter unit
   7.5. magnetic stir plate and stir bars
   7.6. Millipore Pellicon XL Tangential Apparatus
   7.7. peristaltic pump
   7.8. two pieces of ~40 cm long tubing
   7.9. 1 Masterflex ~60 cm long thick wall tubing. (Masterflex 96400).
   7.10. 3 fittings and 1 clamp
   7.11. 3 containers, 500mL
   7.12. 50 mL graduated cylinder
   7.13. 3 cable ties
   7.14. biopure water
8. **Procedure:**
   8.1. **Preparation and Set Up**

Title: Millipore Pellicon XL Tangential Flow Filter SOP

8.1.1. Prepare 0.1M NaOH for cleaning (if needed).
- 8.1.1.1. Using a 1L graduated cylinder, measure 1L of deionized water.
- 8.1.1.2. Transfer water to a 1L flask.
- 8.1.1.3. Weigh 4.0±0.05g of NaOH.
- 8.1.1.4. Transfer NaOH to flask.
- 8.1.1.5. Add magnetic stir bar and stir to dissolve.
- 8.1.1.6. Sterile filter the solution and label container: 0.1M NaOH, [date], [initials], [group number], storage: room temp, disposal: adjust to pH 7 then drain.

8.1.2. Collect two pieces of ~ 40cm long tubing, and one piece of ~60cm long thick wall tubing (Masterflex 96400-16).

8.1.3. If necessary, attach fittings to one end of each tubing and cable tie to secure.

8.1.4. Obtain the Millipore Pellicon XL Tangential Filter from the 2-8°C refrigerator. Note: the best filter composition for protein purification is regenerated cellulose, check for the filter composition on the label.

8.1.5. Take off the caps to the Feed, Retentate and the Permeate 2 port.

8.1.6. Attach the tubing to the Pellicon filter, making sure that the long tubing is placed on the Feed port.

8.1.7. Loosely attach a thumbscrew clamp to the retentate tubing.

8.1.8. Open the rotary assembly latch in the front of the peristaltic pump, place the feed tubing around the top of the rotor assembly and close latch. Secure the tubing in place with the two black clips.

8.1.9. Place the end of the Feed and Permeate tubing into separate 500mL containers and the Retentate tubing into a 50mL graduated cylinder.

8.2. **Flushing**

8.2.1. Fill Feed container with approximately 500mL of biopure water.

8.2.2. Make sure that the speed dial on the peristaltic pump is set at zero and that the CW/CCW switch is in the central "OFF" position.

8.2.3. Place CW/CCW switch to appropriate setting.

8.2.4. Adjust speed dial until the rotor assembly begins to move starting the pump.

8.2.5. Set the retentate flow rate to 30-50 mL/min.

8.2.5.1. To measure the flow rate, collect water for 1 minute. Measure the volume collected in the graduated cylinder to obtain flow rate. Adjust pump speed if necessary and measure again. Once the correct flow rate is obtained, note the speed setting for future reference.
Note: DO NOT CHANGE the speed setting for the remainder of this SOP.

8.2.6. Flush about 150mL of the bipure water through the retentate tube until the pH of the fluid flowing directly from the retentate tubing is near neutral as determined with pH paper.

8.2.7. Tighten the thumb screw clamp on the retentate tube (not completely).

8.2.8. Continue flushing until about 300mL of biopure water are collected in the permeate container and the fluid flowing directly from the permeate tubing is near neutral as determined with pH paper.

8.2.9. Turn off pump and empty feed, permeate and retentate containers into the sink.

Title: Millipore Pellicon XL Tangential Flow Filter SOP

8.3. **Preconditioning**

8.3.1. Place the end of the retentate tube into the feed container with the feed tube to recirculate the material.
8.3.2. Place the permeate tube into the 50mL graduated cylinder.
8.3.3. Fill feed container with 100-200mL of the appropriate buffer (see process SOP).
8.3.4. Turn on the pump and pump until approximately 30-50mL of buffer are collected through the permeate tube.
8.3.5. Turn off pump and empty feed and permeate containers into the sink.

8.4. **Cleaning and Storing**

8.4.1. Loosen the clamp on the retentate tube.
8.4.2. Perform the flushing procedure (step 8.2.6-8.2.9) with biopure water.
8.4.3. Clean with 0.1M NaOH.
8.4.3.1. Place the retentate tube into the same container as the permeate tube. This will be the waste container.
8.4.3.2. Loosen the thumbscrew clamp on the retentate tube.
8.4.3.3. Fill the feed container with 500mL of 0.1M NaOH.
8.4.3.4. Turn on the pump.
8.4.3.5. Collect 250mL of solution in the waste container (containing the retentate and permeate tubes).
8.4.3.6. Turn off the pump.
8.4.3.7. Place retentate tube in the feed container.
8.4.3.8. Turn on the pump.
8.4.3.9. Tighten the thumbscrew on the retentate tube to increase the permeate flow rate.
8.4.3.10. Recirculate the cleaning solution for 30-60 minutes.
8.4.3.11. Turn off the pump off.
8.4.3.12. Neutralize the NaOH in the waste container with HCl and flush down the drain.
8.4.3.13. Carefully remove the tubes, place caps on the attachment fittings and store the device flat at 2-8°C.
8.4.3.14. Rinse the tubing with deionized water before storing.

9. Attachments:

9.1. Figure 1: Pellicon XL with stand
9.2. Figure 2: Set Up

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial release
SCP	2003	
Deb Audino	070505	Put into 2005 SOP format.
Deb Audino	12May06	Added attachments.
Deb Audio	04Apr08	College name change

Title: Millipore Pellicon XL Tangential Flow Filter SOP

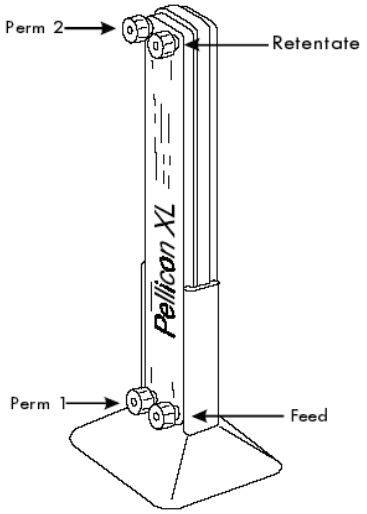


Figure 1: Millipore Pellicon XL Device
(http://www.millipore.com/userguides.nsf/docs/p60085)

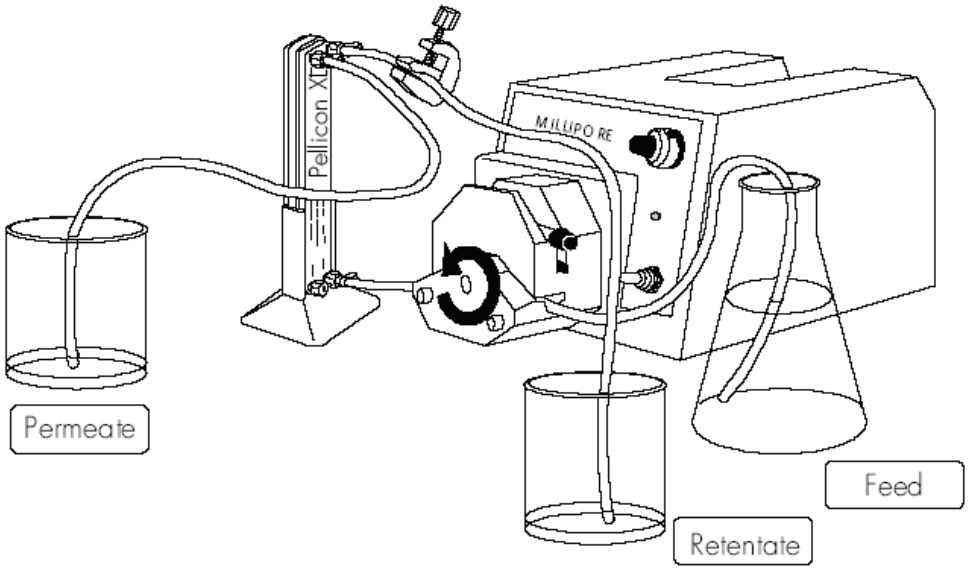


Figure 2: Set Up
(http://www.millipore.com/userguides.nsf/docs/p60085)

Title: Affinity Chromatography of HSA

Approvals:
Preparer:_____Kari Britt_____ Date _____02Jun09_____
Reviewer:_____Bob O'Brien_____ Date _____02Jun09_____

1. **Purpose:**
   1.1. To purify HSA using affinity chromatography.
2. **Scope:**
   2.1. Applies to purifying HSA using Affi-Gel Blue beads and the BioLogic LP system.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Affi-Gel Blue Manufacturer's Instructions
   4.2. pH meter SOP
   4.3. Amicon/Millipore column assembly SOP
   4.4. BioLogic LP SOP
5. **Definitions:**
   5.1. CV: Column Volume; CV = π(L in cm)[(radius of column in cm)^2]
   5.2. L = Length of column (meaning the height of the bead bed)
   5.3. HETP: Height Equivalent to Theoretical Plate; HETP = L/N
   5.4. $N = 5.54\ (t_R/w_{1/2})^2$
   5.5. t_R: retention time
   5.6. $w_{1/2}$: peak width at half height
   5.7. h: Reduced Plate Height; h = HETP/Dp
   5.8. Dp: bead diameter
6. **Precautions:**
   6.1. 2.5mM NaOH is very corrosive. It is extremely damaging to eyes and mucous membranes. It causes burns. Avoid contact with skin. Harmful if swallowed or inhaled.
7. **Materials:**
   7.1. Amicon Vantage-L Biochromatography column and accessories
   7.2. Affi-Gel Blue Gel beads from BioRad (Catalog number: 153-7301)
   7.3. BioRad BioLogic LP System
   7.4. 0.22µm sterile filter units (Nalgene)
   7.5. waste beakers
   7.6. laboratory film, such as Parafilm
   7.7. 1mL syringe
   7.8. ring stand with clamps
   7.9. biopure water
   7.10. Equilibration Buffer A: 20mM Phosphate buffer, pH 7.1
      7.10.1. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)
      7.10.2. Na_2HPO_4-$7H_2O$ (sodium phosphate dibasic, heptahydrate)

Title: Affinity Chromatography of HSA

7.11. Elution Buffer B: 20mM Phosphate buffer, pH 7.1, 1M NaCl
- 7.11.1. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)
- 7.11.2. Na_2HPO_4-$7H_2O$ (sodium phosphate dibasic, heptahydrate)
- 7.11.3. NaCl (sodium chloride)

7.12. Cleaning solution: 2.5mM NaOH (sodium hydroxide)

8. Procedure:

8.1. **Prepare buffers and solutions**

8.1.1. **Buffer A:** Equilibration Buffer, 20mM phosphate, pH 7.1
- 8.1.1.1. Weigh out 0.80g NaH_2PO_4 and place into a 1L container.
- 8.1.1.2. Weigh out 3.60g of Na_2HPO_4-$7H_2O$ and place into the 1L container with the NaH_2PO_4.
- 8.1.1.3. Using a 1L graduated cylinder, measure 1L of deionized water.
- 8.1.1.4. Transfer water to the 1L flask.
- 8.1.1.5. Add magnetic stir bar and stir to dissolve.
- 8.1.1.6. Adjust pH to 7.1
- 8.1.1.7. Filter Sterilize.
- 8.1.1.8. Label as: Buffer A, Equilibration Buffer, 20mM Phosphate, pH 7.1, Store Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.2. **Buffer B:** Elution Buffer, 20mM phosphate, pH 7.1, 1M NaCl
- 8.1.2.1. Weigh 29.2 g NaCl and place into a 500mL container.
- 8.1.2.2. Using a 1L graduated cylinder, measure 500mL 20mM Phosphate buffer, pH 7.1 and transfer to the 500mL container containing NaCl.
- 8.1.2.3. Add magnetic stir bar to the container and stir to dissolve.
- 8.1.2.4. Filter sterilize.
- 8.1.2.5. Label as: Buffer B, Elution Buffer, 20mM Phosphate, pH 7.1, 1M NaCl, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.3. **Cleaning Solution:** 2.5mM NaOH
- 8.1.3.1. Using a 1L graduated cylinder, measure 1L of deionized water.
- 8.1.3.2. Transfer water to a 1L vessel.
- 8.1.3.3. Weigh 0.1g of NaOH.
- 8.1.3.4. Transfer the NaOH to the 1L vessel containing water.
- 8.1.3.5. Add a magnetic stir bar and stir to dissolve.
- 8.1.3.6. Filter sterilize.
- 8.1.3.7. Label as: Cleaning Solution, 2.5mM NaOH, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.4. **Buffer C:** Use the concentrated HSA in 20mM Phosphate buffer, pH 7.1 obtained during upstream processing of HSA followed by diafiltration using tangential flow filtration. Label as: Buffer C, Concentrated HSA in 20mM Phosphate buffer, pH 7.1, Store: 2-8°, Dispose: Drain, [date], [group], [initials].
Note: 20mL of Buffer C (concentrated HSA) will be loaded on to the column, so the volume of Buffer C in the vessel should be at least 30mL to prevent air from infiltrating the column.

Title: Affinity Chromatography of HSA

8.2. **Purge BioLogic LP Sytem with Buffer A and zero the UV monitor per the Biologic LP Chromatography System SOP.**

8.3. **Pour Column per the BioLogic LP Chromatography System SOP.**

8.3.1. Use approximately 5mL of Affi-Gel Blue Gel beads.

8.4. **Attach the column to the BioLogic LP per the BioLogic LP Chromatography System SOP.**

8.5. **Pack Column per the BioLogic LP Chromatography System SOP.**

8.5.1. Place the line for Buffer A into the vessel containing Buffer A, Equilibration Buffer. Cover the vessel opening with a laboratory film, such as Parafilm.

8.5.2. Use Method: Affi Pack

Step 1: 0 to 40min Buffer A 0.5mL/min

8.6. **Determine the HETP and h of the column per the BioLogic LP Chromatography System SOP.**

8.6.1. Use Method Affi HETP:
Step 1: 0 to 30min Buffer A 0.5mL/min

8.6.2. The Dp of the bead is 0.3mm.

8.6.3. The expected HETP is approximately 0.6mm.

8.6.4. The h calculation should be less than 3. If h is greater than 3, the desired product may not bind the column efficiently. In this case it is best to re-pack the column.

8.7. **Run Column per the BioLogic LP Chromatography System SOP.**

8.7.1. Place the lines for Buffers A, B, and C into the vessels containing the appropriate buffer. Cover the vessels with laboratory film.

8.7.2. Use Method: Affi HSA

Step 1:	0 to 80min	Buffer C	0.25mL/min
Step 2:	80 to 120min	Buffer A	0.5mL/min
Step 3:	120 to 160min	Buffer B	0.5mL/min
Step 4:	160 to 200min	Buffer A	0.5mL/min

8.7.3. Collect 1-5mL of the flow through fraction when the first A.U. peak begins to plateau (approximately 40 minutes into the run).

8.7.4. Collect the entire elution fraction when the second A.U. peak BEGINS to appear (approximately 100 minutes into the run).

8.7.5. Store fractions at 2-8^{o}C for SDS PAGE analysis.

8.8. **Clean the Column per the BioLogic LP Chromatography System SOP.**

8.8.1. Place the lines for Buffer A and B into the Cleaning Solution, 2.5mM NaOH.

8.8.2. Use Method: Affi Clean

8.8.2.1. Step 1: 0 to 40min Buffer 50% B 0.5mL/min

8.9. **Clean and Store the System per the BioLogic LP Chromatography System SOP.**

9. Attachments: N/A

Title: Affinity Chromatography of HSA

10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
SCP		Changed from Millipore LC100 system to manual pump system.
Deb Audino	7/2005	Changed from manual pump system to BioLogic LP system.
Deb Audino	08May06	Removed steps associated with equipment operation to simplify the process SOP.
Deb Audino	18Jan08	Decreased flowrate and run time on step 1 for Affi HSA program.
Deb Audino	04Apr08	College name change
Kari Britt	31May09	Added labeling directions, directions for covering vessels with laboratory film, and directions for placing buffer lines into the appropriate buffer. Also made general grammar and formatting edits as needed throughout the document.

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

Record Keeping Standards:

For each step in the batch record: the operator of the task will enter their initials (each operator has their own unique set of initials) and the date in the appropriate section(s) of the batch record. Another operator must initial and date in the appropriate section of the batch record to verify that the task was completed per SOP. No operator will verify their own work at any point. “If you didn’t document it, you didn’t do it!”

Batch records will be completed in blue or black ball point pen ONLY, and must be legible.

Any errors on a batch record will be crossed out with a single line through the error with the initials of the operator and the date. Corrections will be written in next to the crossed out error.

Use the following format to record dates: DDMMMYY. For July 10, 2006 use 10JUL06.

Use the 24 hour clock or “military time” to record time: 3:00pm would be written as 15:00.

Any and all deviations from a protocol or SOP, including abnormal results or retests performed, will be entered into the comments section at the end of each batch record. Be as detailed and specific as possible, include all steps taken before and/or after an abnormal reading, and provide an explanation for any deviations from a step.

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

1. Solution and Buffer Preparation for Tangential Flow Filtration 20mM Phosphate Buffer pH 7.1 0.1M Sodium Hydroxide		
Calibrate pH meter per SOP with commercially prepared standard buffers (pH 7 and pH 4): pH Meter ID #_____ pH 7 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ pH 4 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Weigh 0.80±0.02 grams sodium phosphate monobasic, anhydrous (NaH_2PO_4). Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Weigh 3.6±0.2 grams sodium phosphate dibasic, heptahydrate (Na_2HPO_4-$7H_2O$). Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve sodium phosphate monobasic anhydrous with the sodium phosphate dibasic heptahydrate in approximately 1L of deionized water using magnetic stir bar. Volume of water added: _____ mL	Operator/Date	Verifier/Date
Adjust 20mM Phosphate Buffer to pH 7.1±0.1. pH_____	Operator/Date	Verifier/Date
Sterile Filter solution and label container: 20mM Phosphate Buffer pH 7.1, [date], [initials], [group], storage: room temp, disposal: drain.	Operator/Date	Verifier/Date
Weigh 4.0±0.2 grams of sodium hydroxide (NaOH): Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

Dissolve NaOH in approximately 1L of deionized water using magnetic stir bar. Volume of water added: _____ mL	Operator/Date	Verifier/Date
Sterile filter solution and label container: 0.1M NaOH, [date], [initials], [group number], storage: room temp, disposal: adjust to pH 7 then drain.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
2. Set up, flush, and precondition the tangential flow filtration apparatus.		
Obtain Millipore Pellicon XL Tangential Filter from 2-8°C. Millipore Pellicon XL ID#_____ **Obtain** Millipore peristaltic pump. Pump ID# _____	Operator/Date	Verifier/Date
Flush system per Millipore Pellicon XL Tangential Flow Filter SOP. While flushing, set the flow rate to 30-50ml/min. Note: DO NOT adjust speed dial once the correct flow rate is achieved. Flow Rate: _____ Pump Speed: _____	Operator/Date	Verifier/Date
Check the pH of the system after flushing. pH of the retentate_____	Operator/Date	Verifier/Date
Precondition the system per Millipore Pellicon XL Tangential Flow Filter SOP with 20mM phosphate buffer. Volume of buffer collected:_____mL	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

3. Concentrate and buffer exchange the sample.		
Pour Pichia supernatant into the feed container. Concentrate per Tangential Flow and Diafiltration of HSA SOP. Initial supernatant volume: _____mL Final supernatant volume: _____mL	Operator/Date	Verifier/Date
Buffer exchange the sample per the Tangential Flow and Diafiltration of HSA SOP. After each concentration step is complete, check pH of the retentate. Once the pH of the concentrated retentate is 7.1, TFF is complete Final pH of the concentrated sample (with pH meter):_____	Operator/Date	Verifier/Date
Label container: Filtered Pichia Supernatant, [date], [initials], [group number], storage: 2-8°C, dispose: autoclave and drain. Store for chromatography purification.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
4. Flush, clean and store the tangential flow filtration apparatus.		
Flush the apparatus with biopure water per the Millipore Pellicon XL Tangential Flow Filter SOP.	Operator/Date	Verifier/Date
Clean the apparatus with 0.1M NaOH per SOP until the pH of the retentate is greater than 10. pH of the retentate:_____	Operator/Date	Verifier/Date
If storing unit, leave lines filled with 0.1M NaOH and label unit with status tag: Stored: 0.1M NaOH, [date], [initials].	Operator/Date	Verifier/Date
If not storing unit, flush lines with biopure water until the pH of the retentate is <7.2. Label unit: Cleaned/Rinsed: 0.1M NaOH/biopure water, [date], [initials]. pH of the retentate:_____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

5. Solution and Buffer Preparation for Affinity Chromatography of HSA Buffer A: Equilibration Buffer, 20mM Phosphate, pH 7.1. Buffer B: Elution Buffer, 20mM Phosphate pH 7.1, 1M NaCl Cleaning Solution: 2.5mM NaOH		
Calibrate pH meter per SOP with commercially prepared standard buffers (pH 7 and pH 4): pH Meter ID #_____ pH 7 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ pH 4 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Weigh 0.80±0.02 grams sodium phosphate monobasic, anhydrous (NaH_2PO_4). Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Weigh 3.6±0.2 grams sodium phosphate dibasic, heptahydrate (Na_2HPO_4-$7H_2O$). Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve sodium phosphate monobasic anhydrous with the sodium phosphate dibasic heptahydrate in approximately 1L of deionized water using magnetic stir bar. Volume of water added: _____ mL	Operator/Date	Verifier/Date
Adjust 20mM Phosphate Buffer to pH 7.1±0.1. pH_____	Operator/Date	Verifier/Date
Sterile Filter solution and label as: Buffer A, Equilibration Buffer, 20mM Phosphate, pH 7.1, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

Weigh 29.2 ±0.2 grams NaCl. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve in approximately 500mL of Equilibration Buffer A using magnetic stir bar. Volume of Buffer A added _____ mL	Operator/Date	Verifier/Date
Sterile filter solution and label as: Buffer B, Elution Buffer, 20mM Phosphate, pH 7.1, 1M NaCl, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Weigh 0.10 ±0.02 grams of NaOH. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve in approximately 500mL deionized water using magnetic stir bar. Volume of water added _____ mL	Operator/Date	Verifier/Date
Sterile filter solution and label as: Cleaning Solution, 2.5mM NaOH, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Label the concentrated HSA in 20mM Phosphate buffer, pH 7.1 as: Buffer C, Concentrated HSA in 20mM Phosphate buffer, pH 7.1, Store: 2-8°, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

6. Purge BioLogic LP System, Pour Column and Attach to Biologic LP System		
Calibrate pump if necessary per the BioLogic LP Chromatography System SOP. **Verify** that **1.6mm** tubing is in the pump. Change tubing if necessary. Tubing changed: Yes / No (Circle) If the tubing was changed, adjust the platen and calibrate the pump per BioLogic LP SOP. Platen adjusted: Yes / No (Circle) Pump recalibrated: Yes / No (Circle)	Operator/Date	Verifier/Date
Purge the BioLogic LP system with Buffer A per the Biologic LP Chromatography System SOP.	Operator/Date	Verifier/Date
Place each buffer line into a container filled with Buffer A (Equilibration Buffer).	Operator/Date	Verifier/Date
Zero the UV monitor per the Biologic LP Chromatography System SOP.	Operator/Date	Verifier/Date
Add approximately 5mL of Affi-Gel Blue beads to column per BioLogic LP Chromatography System SOP. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Volume of Affi-Gel Blue added _____ mL	Operator/Date	Verifier/Date
Attach the column to the BioLogic LP per the BioLogic LP Chromatography System SOP. BioLogic LP ID#_____ Amicon Vantage-L-Column ID#_____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

7. Pack the Column and Determine HETP and h		
Pack column per the BioLogic LP Chromatography System SOP using Method: Affi Pack.	Operator/Date	Verifier/Date
Place the line for Buffer A into the vessel containing Buffer A, Equilibration Buffer. Cover the vessel opening with a laboratory film, such as Parafilm.	Operator/Date	Verifier/Date
Determine column volume per the BioLogic LP Chromatography System SOP. CV = π(bed height in cm)(radius of column in cm)^2 Write out CV calculation in this space: Bed Height: _____ Column Volume: _____	Operator/Date	Verifier/Date
Produce chromatogram needed to determine HETP and h per BioLogic LP Chromatography System SOP using Method: Affi HETP. Volume of Elution Buffer B loaded:_____mL	Operator/Date	Verifier/Date
Determine HETP of the column per BioLogic LP Chromatography System SOP and **attach** chromatogram to batch record. Dp = 0.3mm for Affi-Gel Blue beads. Write out HETP and h calculations in this space: HETP value:_____mm h value:_____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

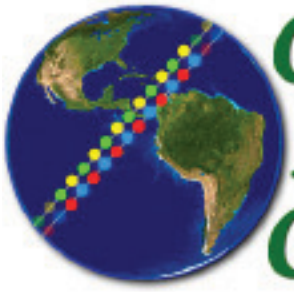
8. Run Column		
Run column per the BioLogic LP Chromatography System SOP using Method: Affi HSA.	Operator/Date	Verifier/Date
Place the lines for Buffers A, B, and C into the vessels containing the appropriate buffer. Cover the vessels with laboratory film.	Operator/Date	Verifier/Date
Store fractions at 2 – 8ºC for SDS PAGE Analysis.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
9. Clean and Store BioLogic LP Chromatography System		
Clean the column per the BioLogic LP Chromatography System SOP using Method: Affi Clean. Use Cleaning Solution, 0.1M NaOH for Buffers A and B.	Operator/Date	Verifier/Date
Clean and store the BioLogic LP Chromatography System per the BioLogic LP Chromatography System SOP. Column Storage (Check one): Left on Biologic System _____ Disconnected and stored at room temp. _____ Disconnected and stored at 2-8°C _____ Disassembled _____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Manufacturing Technician (Downstream)

REF	Key Functions & Tasks (Downstream Manufacturing Technician)
1	**Work in compliance with EH&S.**
1.a	Wear appropriate personal protective equipment.
1.b	Work in controlled environments.
1.c	Participate in emergency drills and emergency response teams.
1.d	Identify unsafe conditions and take corrective action.
1.e	Appropriately and safely access production equipment.
1.f	Handle, label, and dispose of hazardous / biohazard materials.
1.g	Access and utilize MSDS.
1.h	Perform permitting procedures.
1.i	Carries out operations with attention to OSHA and EPA regulations, and other applicable state and federal regulations.
1.j	Keeps work areas clean and safety equipment in order.
1.k	Participate in all company safety training and audits as required.
1.l	Assists with waste treatment operations.
2	**Work in compliance with cGMPs.**
2.a	Assists in environmental monitoring activities.
2.b	Follow SOPs for all operations.
2.c	Records process data and completes batch records as required.
2.d	Maintain equipment logbooks.
2.e	Control and receipt of raw materials.
2.f	Maintain training documentation.
2.g	Maintain equipment and processes in a validated state.
2.h	Working in controlled/classified areas (gowning, aseptic technique).
2.i	Ensure appropriate flow of personnel, equipment, and materials.
2.j	Change control for process, equipment, and documentation.
2.k	Label and apply status to equipment and materials.
2.l	Identify and report exception events and CAPA.
3	**Clean and maintain production areas.**
3.a	Housekeeping / pest control.
3.b	Sanitize and clean of controlled spaces.
3.c	Preparation of cleaning materials and solutions.
3.d	Assist in environmental monitoring for routine and changeover operations.
3.e	Document cleaning.
4	**Maintain effective communication.**
4.a	Deliver shift change update.
4.b	Communicate with coworkers and/or customers to ensure production or service meets requirements.
4.c	Suggest continuous improvements.
4.d	Coordinate with work teams / internal customers.
4.e	Maintain security and confidentiality.
4.f	Respond appropriately to internal auditors and external inspectors.
4.g	Assist in writing, reviewing, and commenting on technical documents.

REF	Key Functions & Tasks (Downstream Manufacturing Technician)
5	**Prepare process materials.**
5.a	Weigh, dispense, and label raw materials for use in production.
5.b	Dispense consumables and intermediates.
5.c	Control and reconcile inventory with enterprise control system (MRP, SAP, manual database).
5.d	Prepare and sterilize buffers and solutions.
5.e	Sample and test buffers and solutions.
5.f	Transfer buffers and solutions to use point.
5.g	Prepare filters for use.
5.h	Prepare, pasteurize / sterilize, and titrate media and feed solutions.
5.i	Manage chromatography resins.
6	**Prepare equipment.**
6.a	Clean CIP vessels, transfer lines, and filter trains .
6.b	Clean COP equipment (or sonicator).
6.c	Depyrogenate components and equipment.
6.d	Sterilize SIP vessels, transfer lines, and sampling ports.
6.e	Perform pressure test.
6.f	Prepare and assemble components and equipment.
6.g	Autoclave components and equipment.
6.h	Perform WFI flush of transfer lines.
6.i	Perform scheduled sanitizations of hoods.
6.j	Prepare and standardize probes and ancillary instruments.
6.k	Prepare, assembly, and integrity test filters.
6.l	Visually inspect equipment.
6.m	Maintain equipment logs and status tags.
6.n	Complete, review and approve equipment process records.
7	**Perform basic manufacturing operations.**
7.a	Perform processes following batch records, validation protocols, and/or SOPs.
7.b	Maintains and controls processes in an automated control environment.
7.c	Record process data.
7.d	Inspect materials at all stages of process to determine quality or condition.
7.e	Participate in the installation, modification, and upgrade of equipment.
7.f	Operate, monitor, and maintain equipment, tools, and workstation.
7.g	Recognize and respond appropriately to atypical events.
7.h	Participate in troubleshooting and root cause analysis of operations.
8	**Perform downstream manufacturing operations.**
8.a	Receive material from upstream processing.
8.b	Separate cells from media using centrifugation or filtration (TFF or depth filtration).
8.c	Perform cell disruption techniques (mechanical or chemical).
8.d	Perform aqueous separations (liquid liquid extraction, precipitation).
8.e	Perform chemical/enzymatic modifications to product.
8.f	Perform normal flow and tangential flow filtration (including microfiltration, ultrafiltration, or diafiltration).
8.g	Prepare chromatography columns (assembly, sanitization, resin packing, evaluation).
8.h	Perform chromatography steps (equilibrate, load, wash, elute, clean, store).
8.l	Perform viral clearance (removal/inactivation) steps.
8.j	Bulk fill purified product.
9	**Perform Sampling.**
9.a	Prepare sample port for aseptic sampling.
9.b	Obtain in-process samples according to batch records or sampling plans.
9.c	Label samples appropriately.
9.d	Record sample collection and distribution (storage and chain of custody)
9.e	Perform in-process chemical and/or microbiological tests.

QC Biochemistry



GLOBAL BIOMANUFACTURING CURRICULUM

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QC Biochemistry

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Title: SDS-PAGE SOP

Approvals:
Preparer:_____Deb Audino_____Date ____03Apr08_____
Reviewer:_____Kari Britt_____Date ____03Apr08_____

1. **Purpose:**
   1.1. To describe the appropriate operating instructions to perform SDS PAGE analysis of proteins samples.
2. **Scope:**
   2.1. Applies to confirming the presence and purity of the two human proteins (tPA and HSA) we have produced and purified in this class.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Invitrogen Novex Gel instructions
   4.2. Novex XCell II Mini-Cell Gel Box Operation SOP
   4.3. gel documentation instrument SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Acrylamide is a neurotoxin. Always wear protective gloves when handling the polyacrylamide gels.
   6.2. Fixative Solution is acidic and flammable. Keep it away from sparks and flames. Dispose in Fixative Hazardous Waste bottle
   6.3. GelCode Blue is harmful. Dispose in GelCode Blue Harzardous Waste bottle.
7. **Materials:**
   7.1. protein samples
   7.2. protein standard, 4mg/mL
   7.3. molecular weight marker (SeeBlue® Plus 2 Pre-stained Standard by Invitrogen is recommended. Catalog number: LC 5925)
   7.4. NOVEX Precast Gel Box and accessories
   7.5. power supply for protein electrophoresis
   7.6. NuPAGE 4-12% Bis-Tris Gels (1.0mm x 10 well)
   7.7. NuPAGE MOPS SDS Running Buffer (20X)
   7.8. NuPAGE Antioxidant
   7.9. NuPAGE SDS Sample Buffer (4X)
   7.10. reducing agent (10X)
   7.11. graduated cylinders (100mL, 250mL, 1L)
   7.12. P20, P100 or P200 Micropipettor and tips, including gel loading tips
   7.13. microfuge Tubes
   7.14. microfuge
   7.15. boiling water bath
   7.16. staining trays
   7.17. rotary shaker

Title: SDS-PAGE SOP

7.18. Fixative Solution
7.19. Pierce GelCode Blue Staining Reagent
7.20. light box
7.21. gel documentation instrument

8. Procedure:

8.1. **Prepare Running Buffers and Fixative Solution if needed.**

8.1.1. **Lower Buffer:** 1X NuPAGE MOPS SDS Running Buffer (1Liter)

8.1.1.1. Place 50mL of 20X NuPAGE MOPS SDS Running Buffer in a 1 Liter graduated cylinder.

8.1.1.2. Gently add 950mL deionized water by running it down the side of the cylinder to make 1 liter of 1X NuPAGE MOPS SDS Running Buffer.

8.1.1.3. Add a stir bar and gently stir.
Note: SDS is a detergent and will foam if mixed vigorously.

8.1.2. **Upper Buffer:** 1X NuPAGE MOPS SDS Running Buffer plus antioxidant (200mL)

8.1.2.1. Add 200mL of 1X NuPAGE MOPS SDS Running Buffer to an appropriate vessel.

8.1.2.2. Add 500µL of NuPAGE Antioxidant.

8.1.2.3. Add a stir bar and gently stir.
Note: SDS is a detergent and will foam if mixed vigorously.

8.1.3. **Fixative Solution** (500mL)

8.1.3.1. In a 500mL bottle, mix together:
250mL 100% Methanol
215mL deionized water
35mL glacial acetic acid

8.1.3.2. Store at 2– 8°C until needed.

8.2. **Prepare Protein Samples.**

Note: Do NOT perform this step with the Molecular Weight Marker.

8.2.1. For all the samples and the standards, combine the following in a sterile microfuge tube:
25µL 4x sample buffer
10µL 10x reducing agent
65µL sample

8.2.2. Mix gently with a pipet by aspirating and dispensing at least 3 times.

8.2.3. Boil for 3-5 minutes.

8.2.4. Remove from boiling water bath.

8.2.5. Pulse all samples and standards in a microfuge for 30 seconds.

8.3. **Prepare Novex Precast Gel Box.**

8.3.1. Assemble gel box according to its SOP.

8.3.2. Place 200mL NuPAGE MOPS SDS Running Buffer (1X) plus antioxidant in the upper buffer chamber (small chamber between 2 gels or the gel and buffer dam).

8.3.3. Fill the lower buffer chamber with approximately 600mL of 1X NuPAGE MOPS SDS Running Buffer (large chamber).

Title: SDS-PAGE SOP

8.4. **Load Samples.**

8.4.1. Using a micropipettor and disposable tips, load 10µL of the Molecular Weight Marker into one well and up to 50µL of each sample into separate wells.

8.4.1.1. Avoid loading samples symmetrically.

8.4.2. Load any empty wells with 15µL of diluted 4X Sample Buffer.

8.4.3. Record order of samples and volumes loaded.

8.5. **Run NOVEX NuPAGE MOPS SDS Precast Gel Box.**

8.5.1. Plug electrophoresis chamber into the gel electrophoresis power supply.

8.5.2. Run gel at 200V for 40 – 60 minutes.

8.5.3. Turn off the power supply when the dye reaches 1cm from the bottom of the gel.

8.6. **Stain and Photodocument the NOVEX NuPAGE MOPS SDS Precast Gel.**

8.6.1. Disassemble gel box per SOP and remove gel from plastic cassette.

8.6.2. Rinse gel box well with DI water. Do not use brushes on the gel box, they scratch the surface. Do not immerse top of gel box or electrical components.

8.6.3. Place gel in staining tray.

8.6.4. Wash gel 3 times for approx. 5 minutes with DI water shaking at room temp.

8.6.5. Add enough Fixative solution to completely cover the gel and fix for approx. 15 minutes shaking at room temp.

8.6.6. Discard Fixative Solution into the Fixative Hazardous Waste bottle

8.6.7. Wash gel 3 times for a minimum of 5 minutes with DI water shaking at room temp.

8.6.8. Add about 50mL of GelCode Blue and stain for 1-24 hours shaking at room temp.

8.6.9. Decant GelCode Blue into GelCode Blue Hazardous Waste bottle.

8.6.10. Wash gel with DI water for 15 minutes to overnight on a shaker

8.6.11. Remove gel from staining tray and place on visible light box

8.6.12. Identify the protein standards and samples and estimate their molecular weights. See Molecular Weight Diagram.

9. Attachments:

9.1. Figure 1: Molecular Weight Marker Diagram

10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
SCP	2003	Changed Coomassie stain to GelCode Blue Stain
Deb Audino	2005	Put into SOP 2005 format
Deb Audino	09May06	Removed dilute protein standards
Deb Audino	04Apr08	College name change

Title: SDS-PAGE SOP

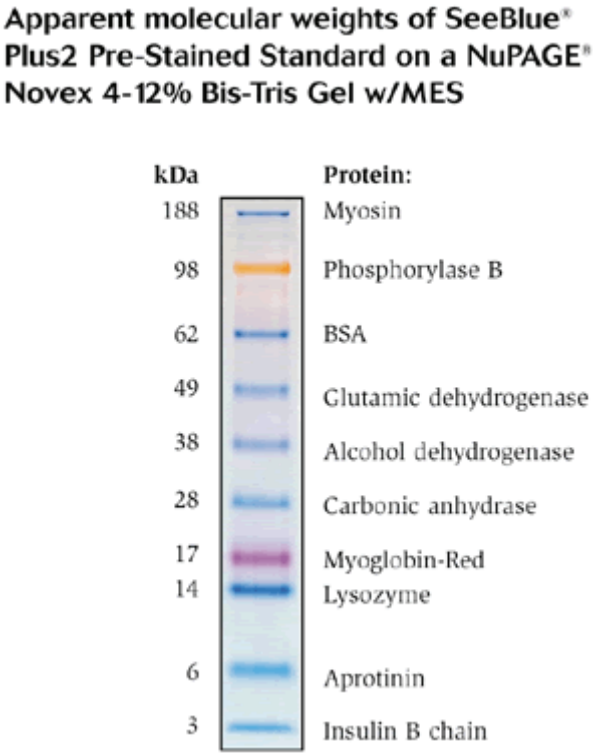


Figure 1: Molecular Weight Marker Diagram

Great Bay Community College
320 Corporate Drive
Portsmouth, NH 03801

Title: Xcell *SureLock* Mini-Cell Gel Box SOP

Approvals:
Reviewer: __ Bob O'Brien _____ Date__08Apr08_____
Reviewer: __ Deb Audino_____Date__08Apr08_____

1. **Purpose:**
   1.1. Assembly and disassembly of the XCell *SureLock* ™ mini-cell gel box.
2. **Scope:**
   2.1. Applies to the assembly and disassembly of the XCell *SureLock*™ mini-cell gel box for use in SDS PAGE.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. XCell *SureLock* ™ Mini-Cell Gel Box Instruction Manual.
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Do not attempt to use the XCell *SureLock*™ mini-cell gel box without the XCell *SureLock* lid.
   6.2. Maximum voltage limit: 500 VDC
   6.3. Maximum power limit: 50 Watts
   6.4. Maximum operating temperature limit: 70°C
   6.5. Acrylamide is a neurotoxin. Always wear protective gloves when handling the polyacrylamide gels.
7. **Materials:**
   7.1. pre-cast gel cassette
   7.2. D.I. (deionized) water
   7.3. running buffer
   7.4. external power supply
   7.5. XCell *SureLock* ™ Mini-Cell
   7.6. buffer core with electrodes
   7.7. cell safety lid with power cords
   7.8. gel tension wedge
   7.9. buffer dam
   7.10. gel knife
8. **Procedure:**
   8.1. **Assembly of the Gel Box**
      8.1.1. Lower the buffer core into the lower buffer chamber so that the negative electrode fits into the opening in the brass plate.
      8.1.2. Cut open gel cassette pouch with scissors, drain away and dispose of the gel-packaging buffer.

Title: Xcell *SureLock* Mini-Cell Gel Box SOP

8.1.3. Handling cassette by its edges only, remove the gel cassette from the pouch and rinse with D.I. water.
8.1.4. **Peel off the tape** covering the slot on the back of the gel cassette.
8.1.5. In one fluid motion, carefully remove comb from cassette.
Note: Do not twist comb, pull straight out or damage to wells may occur.
8.1.6. Use a pipette to gently wash the cassette wells with running buffer, invert the gel and shake gently to remove buffer. Repeat twice.
8.1.7. Fill the sample wells with running buffer. Be sure to remove any bubbles from cassette wells.
8.1.8. Insert the gel cassette into the lower buffer chamber to create the upper buffer chamber.
8.1.8.1. If running only one gel, place the gel in front of the buffer core with the shorter (notched) side of the cassette facing in toward the core. Place the buffer dam behind the core. **Do not discard buffer dam.**
8.1.8.2. If running two gels, place one cassette in front of the buffer core and one cassette behind the core, making sure that the shorter (notched) sides are facing in towards the core.
8.1.9. Slide Gel Tension Wedge into the lower buffer chamber behind the buffer dam (or behind second gel) with the tapered end pointing up. See Figure 2.
8.1.10. Pull forward (toward the front of the unit) on the Gel Tension Lever until lever comes to a firm stop and the gels or gel/buffer dam appear snug against the buffer core. See Figure 2.

8.2. **Run the gel.**
8.2.1. Load and run the gel per the SDS-PAGE SOP

8.3. **Disassembly of the XCell *SureLock*™ Mini-Cell Gel Box**
8.3.1. Upon completion of the run, turn off the power and disconnect the electrode cords from the power supply.
8.3.2. Remove the lid.
8.3.3. Unlock the Gel Tension Lever by pushing the lever toward the back of the unit.
8.3.4. Remove gel cassette from the assembly. Handle gel cassette by the edges.
8.3.5. Lay the gel cassette on top of a lab towel, with the shorter plate on top. Allow one side to hang approximately 1 cm over the side of the bench top.
8.3.6. Insert the gel knife between the two plates. See **Figure 3**. (HINT: It may be easier to start with the corner.)
8.3.7. Twist the handle to separate the plates. You will hear a cracking sound which means you have broken the bonds which hold the plates together.
8.3.7.1. Do not push the knife forcefully between the cassette plates or the gel may be cut into and damaged.
8.3.8. Rotate the cassette and repeat steps 8.3.6. and 8.3.7. until the two plates are completely separated.
8.3.9. Using hands only and being very careful not to rip the gel, gently remove and discard the top plate, allow the gel to remain on the bottom plate.

Title: Xcell *SureLock* Mini-Cell Gel Box SOP

8.3.10. Holding the cassette plate over a container with the gel facing downwards gently push the gel knife into the slot at the bottom of the cassette, until the gel peels away from the plate.

8.3.11. If the gel is not easily removed, rinse with D.I. water from a squirt bottle inserted gently between the plate and the gel.

8.3.12. Cut the lip off the bottom of the gel (If needed).

8.3.13. Discard running buffer and rinse gel box well with deionized water. Do not use brushes. Do not immerse top of gel box or electrical components.

8.4. **Stain the gel.**

8.4.1. Stain the gel per the SDS-PAGE SOP.

9. Attachments:

9.1. Figure 1: Gel Box Parts

9.2. Figure 2: Assembled Gel Box Side View

9.3. Figure 3: Opening a Gel Cassette

10. History:

Name	Date	Amendment
Katrice Jalbert	030106	Initial Release
Bob O'Brien	08Apr08	Update the date format, change college name and remove outline of text boxes.

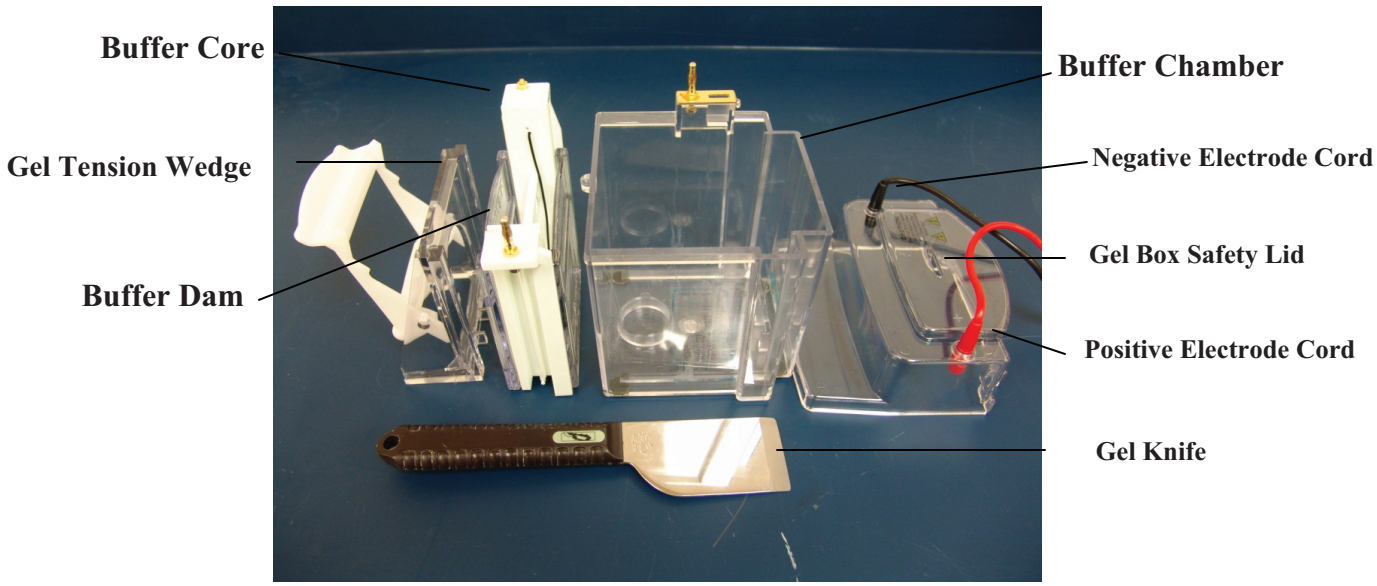


Figure 1: Gel Box Parts

Title: Xcell *SureLock* Mini-Cell Gel Box SOP

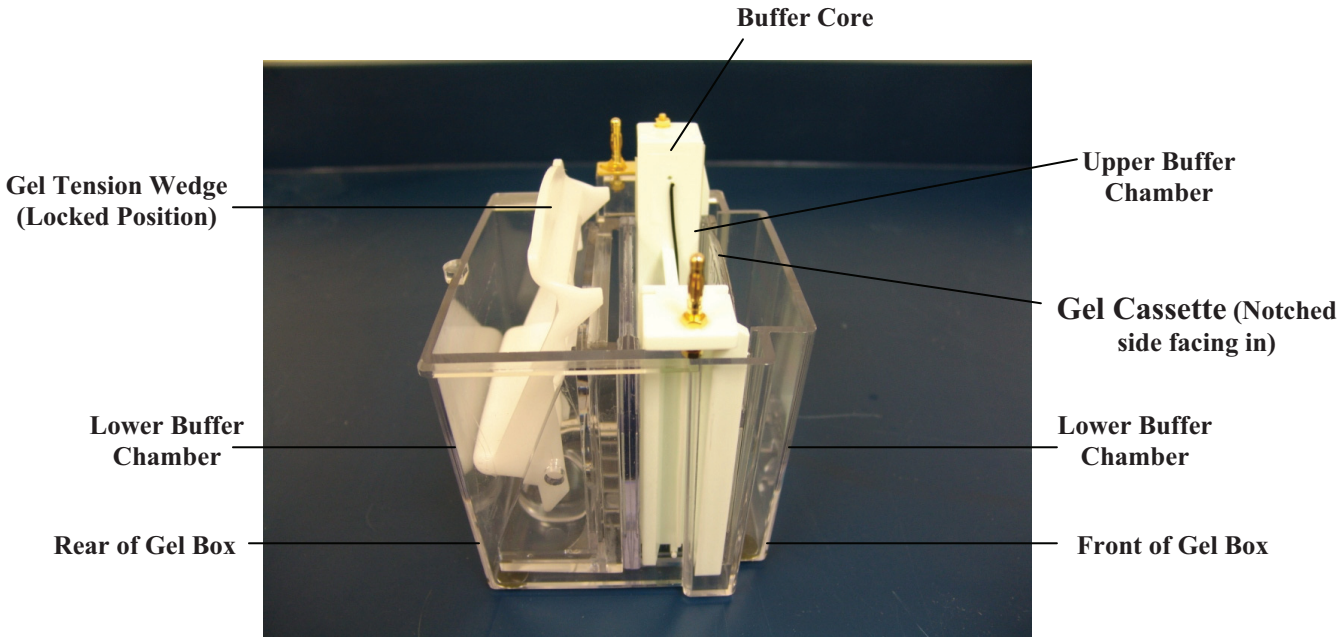


Figure 2: Assembled Gel Box Side View

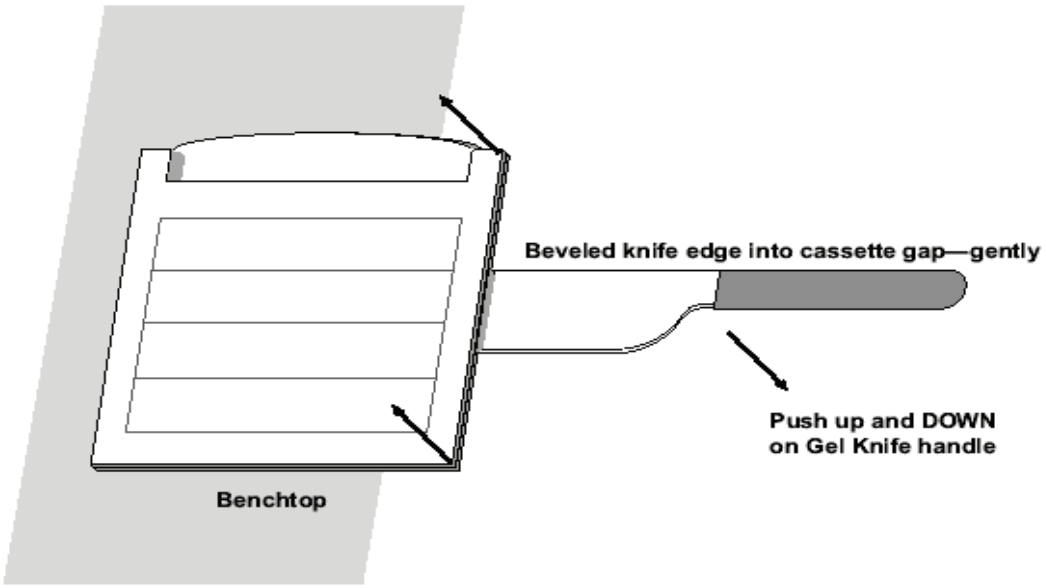


Figure 3: Opening a Gel Cassette

Great Bay Community College
320 Corporate Drive
Portsmouth, NH 03801

Title: tPA ELISA SOP

Approvals:
Preparer:_____Kari Britt_____ Date _____ 09Jun09_____
Reviewer:_____Bob O'Brien_____ Date _____ 09Jun09_____

1. **Purpose:**
   1.1. To determine the concentration of tPA in a sample.
2. **Scope:**
   2.1. Applies to determining the concentration of tPA in a sample using the IMUBIND tPA ELISA kit from American Diagnostica.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. IMUBIND tPA ELISA kit instructions
   4.2. plate reader SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Plasma is of human origin and should be treated as Biosafety Level 2. Dispose of waste in biohazard containers.
7. **Materials:**
   7.1. IMUBIND tPA ELISA kit from American Diagnostica (cat# 860)
      7.1.1. Microtest strips coated with anti-tPA IgG coat and non- immune IgG
      7.1.2. PET buffer
      7.1.3. tPA depleted plasma
      7.1.4. tPA antigen standard plasma
      7.1.5. Conjugate -HRP labeled anti-tPA Fab fragments
   7.2. TMB (3, 3', 5, 5' – Tetramethylbenzidine) substrate
   7.3. disposable reagent reservoirs or weigh boats
   7.4. micropipettors (P-20, P-100 or 200, multichannel) and tips
   7.5. lab towels
   7.6. microfuge tubes
   7.7. microtiter plate reader operable at 630nm
8. **Procedure:**
   8.1. **Sample and Reagent Preparation**
      8.1.1. **PET buffer:**
         8.1.1.1. Dissolve the contents of the PET-buffer vial in 1L ± 50mL of water.
         8.1.1.2. Stir until dissolved, approximately 15 minutes.
         8.1.1.3. Label as: PET buffer, Store: 2-8°, Dispose: Drain, [Date], [Inititials].
      8.1.2. **Prepare Detection Antibody Conjugate:**
      8.1.3. Dilute the 100X Detection Antibody Conjugate with PET buffer according to the following instructions.

Title: tPA ELISA SOP

8.1.3.1. Determine the total number of samples and standards that will be assayed.
8.1.3.2. Determine the final volume of diluted antibody conjugate by multiplying the total number of samples and standards by 55µl.
8.1.3.3. Determine the volume of 100X Detection Antibody Conjugate to use by dividing the final volume of diluted antibody conjugate by 100.
8.1.3.4. Determine the volume of PET buffer to use by subtracting the volume of 100X Detection Antibody Conjugate from the final volume of dilute antibody conjugate.
8.1.3.5. Place the volume of PET buffer needed into a test tube.
8.1.3.6. Add the volume of 100X Detection Antibody Conjugate to the PET buffer. Be sure to dispense the 100X Detection Antibody Conjugate below the surface of the PET buffer and to rinse the inside of the pipet tip by pipetting up and down several times with the tip below the surface of the PET buffer.
8.1.3.7. Close the top of the test tube securely and invert several times to mix.
8.1.3.8. Label the test tube as: Diluted Antibody Conjugate in PET buffer for tPA ELISA, Store: 2-8°C, Dispose: Drain, [Date], [Initials].

8.1.4. **tPA Antigen standard:**

8.1.4.1. Aseptically add 0.5mL of ultrapure water to a vile containing tPA Plasma Standard (30ng/mL).
8.1.4.2. Aseptically add 0.5 mL of ultrapure water to a vile containing tPA Depleted Plasma Standard (0ng/mL).
8.1.4.3. Agitate both vials gently for 5 minutes.
8.1.4.4. Mix the tPA Plasma Standard and the tPA Depleted Plasma Standard in microfuge tubes according to the table below.

Concentration ng/mL	tPA Plasma Standard µL	tPA depleted plasma µL
0	0	30
10	10	20
20	20	10
30	30	0

8.1.4.5. Label the tubes as: tPA standard for ELISA, [Concentration], Store: -20°C, Dispose: Drain, [Date], [Initials].

8.1.5. **CHO samples:**

8.1.5.1. If not done already, centrifuge the samples to remove cells from the media and remove the supernatant to a new tube. Use the supernatant in the assay. This step is not necessary if the cells were removed while performing a previous SOP or if the sample has been eluted from a chromatography column.

Title: tPA ELISA SOP

8.1.5.2. In a new microfuge tube, dilute the samples 1:10 by combining 10µl of sample with 90µl PET buffer.

8.2. **Assay**

8.2.1. Equilibrate all reagents to room temperature before use.

8.2.2. Obtain the number of wells needed for the total number of standards and samples that will be assayed.

8.2.3. Reconstitute wells by adding 50µL ± 1µL of PET buffer to each well. Cover the wells with a laboratory film such as Parafilm, and agitate gently for 3-5 minutes at room temperature.

8.2.4. Add 20µL ± 1µL of each tPA standard and sample to individual wells. Mix gently with the pipettor by aspirating and dispensing 3 times.

8.2.5. Record positions of the standards and samples.

8.2.6. Cover the ELISA wells with laboratory film, and incubate for approximately 1 hour at room temperature while agitating gently.

8.2.7. Add 50µL ± 1µL of the diluted antibody conjugate to the wells. Mix gently with the pipettor by aspirating and dispensing 3 times.

8.2.8. Cover the ELISA wells with a clean sheet of laboratory film and incubate for 15 minutes at room temperature while agitating gently. This will label the bound tPA.

8.2.9. Discard the contents of the wells by turning the wells upside down on a laboratory towel and tapping several times.

8.2.10. Wash the wells four times with PET buffer as follows:

8.2.10.1. Fill each well with approximately 200µl of PET buffer.

8.2.10.2. Turn the wells upside down onto a laboratory towel and tap several times to remove the PET buffer.

8.2.11. After the fourth wash it is VERY important to tap the wells dry until all visible fluid is removed from the wells.

8.2.12. Add 100µL± 1µL TMB substrate solution to each well. Tap the wells gently (right side up) to ensure that the TMB solution settles at the bottom of the wells.

8.2.13. Cove the wells with a clean sheet of laboratory film and incubate at room temperature for 15-60minutes while agitating gently.

8.2.14. Measure the absorbance at 630nm per plate reader SOP.

8.3. **Generate a Standard Curve and Calculate Results**

Note: For detailed directions on how to generate a standard curve using Microsoft's Excel 2007 see section 9 (Attachments). Other appropriate software programs may be used to generate the standard curve.

8.3.1. Plot absorbance at 630nm against the 0, 10, 20, and 30 ng/mL standards.

8.3.2. Fit a linear trendline through the points.

8.3.3. Include the R-squared value and linear equation on the graph.

8.3.4. Use the equation to calculate the concentration of tPA in the wells.

8.3.5. Calculate the concentration of tPA in the culture media by multiplying by the dilution factor of 10.

9. Attachments:

9.1. Figure 1: Standards dilution table located in section 8.2.6.

Title: tPA ELISA SOP

9.2. Directions for generating a standard curve using Microsoft's Excel 2007

9.2.1. Open a new spread sheet in Excel 2007.

9.2.2. Enter the concentration data (X axis) from top to bottom into a column starting with 0 and ending with the number value of the highest concentration.

9.2.3. Enter the corresponding abosorbance value (Y axis) generated by the plate reader in the column directly to the right of the column used to enter the concentration data. For example:

X-value	Y-value
ng/mL	Absorbance
0	0
10	0.092
20	0.179
30	0.320

Note: Enter as many standard concentration values as were used in the assay.

9.2.4. Highlight the cells containing number values only.

9.2.5. Click on the "Insert" tab.

9.2.6. Click on "Scatter" in the "Charts" section.

9.2.7. Choose the chart-type at the top of the left column called "Scatter with only Markers" when you mouse over the choice. The chart will appear.

9.2.8. Right click on one of the data points and choose "Add Trendline…". The "Trendline Options" dialog box will appear.

9.2.9. Select "Linear" and check off "Display Equation on chart" and "Display R-squared value on Chart".

9.2.10. Click on "Close". The line, equation and R-squared value will appear in the chart.

9.2.11. To calculate the concentration of tPA in the sample, substitute the absorbance value for "y" in the equation and solve for "x" and multiply by the dilution factor of 10.

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial release
Deb Audino	07Jul05	Put into 2005 SOP format. Added a 1:5 dilution of samples. Changed substrate to TMB
Deb Audino	04Nov05	Changed dilution process for the conjugate because the kit was changed. Increased the dilution of the samples to 1:10 from 1:5
Deb Audino	04Apr08	College name change , format history update
Kari Britt	09Jun09	Added directions for making a standard curve using Microsoft Excel 2007.

Title: tPA Activity Assay SOP

Approvals:
Preparer:_____Deb Audino_____ Date:_____03Apr08_____
Reviewer:_____Bob O'Brien_____ Date: _____03Apr08_____

1. **Purpose:**
   1.1. To measure tPA activity.
2. **Scope:**
   2.1. To measure tPA activity from cultured cells or purified fractions using the Spectrozyme substrate.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to informed the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. pH meter SOP
   4.2. incubator SOP
   4.3. plate reader SOP (optional)
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. pH meter
   7.2. 37ºC incubator
   7.3. plate reader with 450nm filter (optional)
   7.4. 250mL flask
   7.5. 100mL graduated cylinder
   7.6. 125mL bottle
   7.7. 1 and 10mL pipets and pump
   7.8. 10mL tube
   7.9. 1X Tris-Imidazole buffer with pH 8.4
      7.9.1. 4.04 g tris-base
      7.9.2. 2.27g imidazole
      7.9.3. 13.65g NaCl (sodium chloride)
   7.10. microtiter strips
   7.11. laboratory film such as Parafilm
   7.12. 10µM SPECTROZYME® substrate from American Diagnostica (Catalog number: 444)
   7.13. 10µL (0.04 µg/µL) tPA
   7.14. 1N HCl (or any hydrochloric acid solution for adjusting pH)
8. **Procedure:**
   8.1. **Solution Preparation**
      8.1.1. **10X Tris-Imidazole, pH 8.4.**
         8.1.1.1. Weigh and combine the following chemicals in a 250mL flask:
            4.04g tris-base

Title: tPA Activity Assay SOP

2.27g imidazole
13.65g NaCl (sodium chloride)

8.1.1.2. Add 94mL deionized water.
8.1.1.3. Add 1N HCl (Hydrochloric acid) drop-wise until pH of solution is 8.4.
Note: 1N HCl is recommended, but it is acceptable to use any hydrochloric acid solution approved by the instructor for pH adjustment.
8.1.1.4. Transfer to a 100mL graduated cylinder.
8.1.1.5. Bring volume up to 100mL with deionized water.
8.1.1.6. Transfer to a 125mL bottle.
8.1.1.7. Store at room temperature.

8.1.2. **1X Tris-Imidazole , pH 8.4**
8.1.2.1. Using 1mL and 10mL pipets, combine 1mL of 10X Tris-Imidazole, pH 8.4 and 9mL distilled water in a 10mL tube.
8.1.2.2. Store at room temperature.

8.1.3. **Spectrozyme (5nmol/mL)**
8.1.3.1. Add 2mL deionized water to lyolphilized 10 µMoles SPECTROZYME®.
8.1.3.2. Be sure that the powder is fully dissolved by inverting the bottle several times.
8.1.3.3. Storage: Reconstituted substrate may be stored for 1 week at room temperature, 2 months at 2-8ºC, or up to 6 months at –20ºC (Aliquot and freeze. Do not submit to freeze-thaw cycles).

8.2. **Assay**
8.2.1. Add 80 µL 1X Tris-Imidazole to each well
8.2.2. Add 20µL of 5nmol/mL SPECTROZYME® substrate to each well.
8.2.3. Prepare control wells.
8.2.3.1. Positive (+) Control Well: add 10 µL tPA (0.04 µg/µL) into positive (+) well.
8.2.3.2. Negative (-1) Control Well: add 10 µL Tris-Imidazole buffer into negative (-1) well.
8.2.3.3. Negative (-2) Control Well (only if testing a culture sample): add 10µl media without cells into negative (-2) well.
8.2.4. Prepare sample wells.
8.2.4.1. Place 10µL of each sample into their specified well.
8.2.5. Gently shake microtiter strip well to mix reagents.
8.2.6. Cover the strip with laboratory film.
8.2.7. Incubate wells in 37°C incubator for 1-24 hours.
8.2.8. Remove from incubator and observe tubes. Positive tubes should turn yellow while negative tubes should stay clear.
8.2.9. Optional: Read wells in a plate reader set at 450nm.

9. Attachments: N/A

Title: tPA Activity Assay SOP

10. History:

Name	Date	Amendment
Christopher Cotter Amanda Marshall	01 Jul04	Initial release
Deb Audino	01Jun05	Put into 2005 SOP format and reduced volume of assay.
Deb Audino	04Nov05	Added catalog number of Spectrozyme. Edited adding reagents to wells to clarify the section
Deb Audino	04Apr08	Updated history format. College name change

Title: HSA ELISA SOP

Approvals:
Preparer:_____Bob O'Brien_____ Date ____01Apr09_____
Reviewer:_____Kari Britt_____ Date ____01Apr09_____

1. **Purpose:**
   1.1. To detect Human Serum Albumin (HSA) via Enzyme Linked Immunosorbent Assay (ELISA) and quantify the concentration of HSA in each sample.
2. **Scope:**
   2.1. To detect and quantify the Human Serum Albumin concentration of a given sample using the Human Albumin ELISA Quantitation Set by Bethyl Laboratories, Inc.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Human Albumin ELISA Quantitation Set manual
   4.2. plate reader SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Albumin standards are of human origin and should be treated as Biosafety Level 2. Dispose of waste in biohazard containers.
   6.2. Do not expose TMB Substrate solution to glass, foil or metal. Do not use if solution is blue.
7. **Materials:**
   7.1. Human Albumin ELISA Quantitative set from Bethyl Laboratories (cat #: E80-129)
      7.1.1. ELISA Coating buffer (cat# E107)
      7.1.2. ELISA Wash solution (cat# E106)
      7.1.3. ELISA Blocking buffer (cat# 104)
      7.1.4. Sample/ Conjugate Diluent (ELISA Blocking Buffer + Tween 20)
      7.1.5. 10% Tween 20 (cat#E108)
      7.1.6. Enzyme substrate , TMB (cat# E102)
      7.1.7. 96 well plates
      7.1.8. Human Albumin Standards
   7.2. micropipettors (P-100 or P-200) and tips
   7.3. biopure water
   7.4. paper towels
   7.5. containers to prepare buffers
   7.6. containers to prepare reagents
   7.7. microfuge tubes
   7.8. micro titer plate reader operable at 450 nm
   7.9. 0.18M H_2SO_4 (sulfuric acid)

Title: HSA ELISA SOP

8. Process:

8.1. **Assay Preparation**

8.1.1. Equilibrate all reagents to room temperature before use.

8.2. **Reagent preparation:**

8.2.1. **Wash Solution Buffer**: Tris buffer saline with Tween 20 (cat # E106)

8.2.1.1. Dilute contents of Tris buffer saline with Tween 20 packet in 1L of ultra pure water in an appropriate vessel. Mix thoroughly until the contents go into solution.

8.2.1.2. Label the vessel as: Wash Solution, Tris buffer saline with Tween 20, Store: Room Temperature, Dispose: Drain, [Date], [Initials].

8.2.2. **Blocking Buffer:** Tris buffer saline with 1% BSA. (cat# E104)

8.2.2.1. Dilute contents of Tris buffer saline with 1% BSA packet in 1L of ultra pure water in an appropriate vessel. Mix thoroughly until the contents go into solution.

8.2.2.2. Label vessel as: Blocking Buffer, Tris buffered saline with 1% BSA, Store: 2-8°C, Dispose: Drain, [Date], [Initials].

8.2.3. **Sample/Conjugate Diluent**

8.2.3.1. Combine 500mL of the blocking buffer with 2.5mLof 10% Tween in an appropriate vessel.

8.2.3.2. Label vessel as: Sample/Conjugate Diluent, Tris buffered saline with 1% BSA and 10% Tween, Store: 2-8°C, Dispose: Drain, [Date], [Initials].

8.2.4. **Coating Solution**

8.2.4.1. Break apart the Coating Solution gel capsule and pour contents into 100mL of ultrapure water in an appropriate vessel.
Note: Do not place capsule into the water without breaking it apart. The gelatin from the capsule interferes with the binding of the coating antibody to the plate.

8.2.4.2. Label vessel as: Coating Solution for HSA ELISA, Store: 2-8°C, Dispose: Drain, [Date], [Initials].

8.2.5. **Diluted Coating Antibody Buffer**

8.2.5.1. Dilute 1µL of affinity purified antibody (A80-129A) to 100µL Coating solution buffer for each well to be coated. (Example: for 96 wells, dilute 96µL antibody to 9600µL (9.6mL) ultrapure water).
Note: It is better to mix excess solution than to not have enough. Therefore, for the above example it would be better to add 100µL antibody to 10mL ultrapure water.

8.2.5.2. Place solution in appropriate sized tube. Label tube as: Diluted Coating Antibody, Store: 2-8°C, Dispose: Biohazard waste container, [Date], [Initials].

8.2.6. **Standard Serial Dilutions**

Note: Refer to Figure 1: Standards Dilution Table, on the following page, at the end of this section.

8.2.6.1. Label nine (9) test tubes, one for initial dilution and one for each standard curve point as follows:

Title: HSA ELISA SOP

1. HSA Standard Initial Dilution (15mL test tube)
2. HSA 400ng/mL (15 mL test tube)
3. HSA 200 ng/mL (2mL test tube)
4. HSA 100 ng/mL (2mL test tube)
5. HSA 50 ng/mL (2mL test tube)
6. HSA 25 ng/mL (2mL test tube)
7. HSA 12.5ng/mL (2mL test tube)
8. HSA 6.25ng/mL (2mL test tube)
9. HSA 0 ng/mL, Blank (2mL test tube)

8.2.6.2. **HSA Standard Initial Dilution:** Prepare initial dilution of the 10,000 ng/mL by diluting 5µL of Human Reference Serum (RS10-110-3) with 12.5 mL of Sample/Conjugate Diluent. Mix well by closing the tube securely and inverting several times.

8.2.6.3. Pipette 2.4mL of Sample/Conjugate Diluent into the 15mL test tube labeled 400ng/mL.

8.2.6.4. Pipette 500µL of Sample/Conjugate Diluent into all the other standard tubes: 200 ng/mL, 100 ng/mL, 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL and a 0 ng/mL.

8.2.6.5. Pipette 100µL from the HSA Standard Initial Dilution tube into the 400ng/mL tube.
Note: Wipe excess antibody/ analyte solution from pipette tips between tubes when making dilutions.

8.2.6.6. Pipette 500µL from the 400ng/mL tube into the 200ng/mL tube.

8.2.6.7. Pipette 500µL from the 200ng/mL tube into the 100ng/mL tube.

8.2.6.8. Pipette 500µL from the 100ng/mL tube into the 50ng/mL tube.

8.2.6.9. Pipette 500µL from the 50ng/mL tube into the 25ng/mL tube.

8.2.6.10. Pipette 500µL from the 25ng/mL tube into the 12.5ng/mL tube.

8.2.6.11. Pipette 500µL from the 12.5ng/mL tube into the 6.25ng/mL tube.

8.2.6.12. Do not pipette into the 0ng/mL tube. Use Sample/conjugate diluents only as the blank.

Standard (Std.)	ng/mL	RS10-110-3 (25 mg/mL Human Albumin)	Sample/Conjugate Diluent
Initial	10,000	5µL	12.5mL
1	400	100µL from initial	2.4mL
2	200	500µL from std. 1	500µL
3	100	500µL from std. 2	500µL
4	50	500µL from std. 3	500µL
5	25	500µL from std. 4	500µL
6	12.5	500µL from std. 5	500µL
7	6.25	500µL from std. 6	500µL
8	0	Blank	500µL

Figure 1: Standards Dilutions Table

Title: HSA ELISA SOP

8.2.7. **Dilute HRP Detection Antibody** (cat # A80-129P)

8.2.7.1. Place 12mL of ultrapure water in a 15mL test tube.

8.2.7.2. Dispense 1µL of HRP detection antibody into the 12mL of water.
Note: Be sure to dispense the antibody below the surface of the water. After dispensing pipette up and down several times to rinse the inside of the pipette tip.

8.2.7.3. Close the tube securely and invert several times to mix.

8.2.7.4. Label the tube as: HRP Detection Antibody Solution, 1:12,000, Store: 2 8°C, Dispose: Drain, [Date], [Initials].

8.2.8. **TMB**

Note: Do not use glass pipettes to measure TMB substrate reagents and do not use the TMB if it is blue at any point before adding to the ELISA plate.

8.2.8.1. Prepare the TMB substrate solution in a test tube by mixing equal volumes of the two individual substrate reagents based on the amount of wells used. (Example: For 96 wells and 100µL per well a minimum of 9.6mL will be needed. Therefore, make 10mL of TMB substrate by mixing 5mL of each reagent).

8.2.8.2. Label container containing the mixed TMB substrate as: TMB, Hydrogen Peroxide, Store: 2-8°C and protect from light, Dispose: Drain, [Date], [Initials].

8.2.8.3. Wrap test tube with aluminum foil to block light and refrigerate until needed.
Note: Aluminum foil should not be used to cover the ELISA plate during the reaction.

8.2.9. **Stop Solution**

8.2.9.1. Prepare a 20-100mL solution of 2M sulfuric acid (H_2SO_4) and label as: Stop Solution, 2M sulfuric acid (H_2SO_4), Store: acid cabinet, room temperature, Dispose: neutralize pH then drain, [Date], [Initials].

8.3. **Assay**

8.3.1. **Coating wells**

8.3.1.1. Equilibrate all reagents to room temperature before use.

8.3.1.2. Obtain an ELISA plate.

8.3.1.3. Dispense 100µL of diluted coating antibody to each well that will be used (Extra wells can be coated if excess coating antibody solution remains after the minimum number of wells has been coated.).

8.3.1.4. Incubate at room temperature (20-25°C) for at least 60 minutes**.**

8.3.1.5. Remove the coating antibody solution mixture by turning the plate upside down on a lab towel and tapping the liquid out on to the towel several times.

8.3.1.6. Wash plate per plate washing direction (step 8.3.2.).

Title: HSA ELISA SOP

8.3.2. **Plate Washing**

8.3.2.1. Rinse the wells five times with ELISA Wash solution as follows: Fill each well with ELISA Wash solution (approximately 200µL). Remove the ELISA Wash solution by aspirating with a pipette.

8.3.2.2. Repeat step 8.3.2.1 four additional times.

8.3.2.3. After the fifth wash, blot the plate dry by turning it upside down on a lab towel and tapping several times, to remove visible liquid.
Note: Visible liquid should be removed from the wells, but the operator should also minimize lag time between plate washing and the following assay step to ensure that the plate does not completely dry out during the assay.

8.3.3. **Blocking**

8.3.3.1. Dispense 200µL of blocking solution into each well.

8.3.3.2. Cover the ELISA plate with laboratory film such as Parafilm.

8.3.3.3. Incubate for 30 minutes at room temperature.

8.3.3.4. Wash plate five times per plate washing procedure (step 8.3.2).

8.3.4. **Adding samples and standards**

8.3.4.1. If not done already, centrifuge the samples to remove cells from the media and remove the supernatant to a new tube. Use the supernatant in the assay. This step is not necessary if the cells were removed while performing a previous SOP or if the sample has been eluted from a chromatography column.

8.3.4.2. Add100µL of standard or sample to appropriate wells.

8.3.4.3. Record positions of standards and samples.

8.3.4.4. Cover the ELISA plate with a new sheet of laboratory film.

8.3.4.5. Incubate for 60 minutes at room temperature.

8.3.4.6. Wash plate five times per plate washing procedure (step 8.3.2).

8.3.5. **Adding diluted HRP detection antibody**

8.3.5.1. Add 100µL of diluted HRP detection antibody to each well.

8.3.5.2. Cover the ELISA plate with a new sheet of laboratory film.

8.3.5.3. Incubate for 60 minutes at room temperature.

8.3.5.4. Wash plate five times per plate washing procedure (step 8.3.2).

8.3.6. **Adding TMB Enzyme**

8.3.6.1. Add 100µL of TMB substrate solution to each well. Take care not contaminate the TMB.
Note: Do not expose TMB or ELISA plate wells to glass, aluminum foil, or metal. Also, if the TMB substrate solution is blue before adding to the plate, DO NOT USE IT!

8.3.6.2. Cover the ELISA plate with a new sheet of laboratory film.

8.3.6.3. Develop in a dark room (or area not exposed to light) for approximately 15 minutes.

8.3.6.4. After 15 minutes stop reaction by adding 100µL of stop solution to each well. With the bottom of the plate laying flat on a hard surface, swirl the plate gently to mix solution.

Title: HSA ELISA SOP

Note: Wells with blue solution should turn from blue to yellow after adding the stop solution.

8.3.6.5. Wipe the underside of the plate with a lab tissue.

8.3.7. **Evaluating the ELISA Plate**

Note: The plate must be evaluated on the plate reader within 30 minutes of stopping the reaction.

8.3.7.1. Measure the absorbance of the wells at 450nm per plate reader SOP.

8.3.8. **Generate a Standard Curve and Calculate Results**

Note: For detailed directions on how to generate a standard curve using Microsoft's Excel 2007 see section 9 (attachments). Other appropriate software programs may be used to generate the standard curve.

8.3.8.1. Plot absorbance at 450nm against the standard concentrations.

8.3.8.2. Fit a trend line through the points.

8.3.8.3. Include the R-squared value and linear equation on the graph.

8.3.8.4. Use the equation to calculate the concentration of HSA in the samples.

Note: If sample absorbance values are higher than the range encompassed by the standard curve, they will need to be diluted and the ELISA assay repeated (A 1:10 dilution is recommended in this case.). After calculating the concentration using the equation, multiply by the dilution factor to get the actual concentration.

9. Attachments:

9.1. Figure 1: Standards dilution table located in section 8.2.6.

9.2. Directions for generating a standard curve using Microsoft's Excel 2007

9.2.1. Open a new spread sheet in Excel 2007.

9.2.2. Enter the concentration data (X axis) from top to bottom into a column starting with 0 and ending with the number value of the highest concentration.

9.2.3. Enter the corresponding absorbance value (Y axis) generated by the plate reader in the column directly to the right of the column used to enter the concentration data.

For example:

X-value	Y-value
ng/mL	Absorbance
0	0
6.25	0.092
12.25	0.179
25	0.320

Note: Enter as many standard concentration values as were used in the assay.

9.2.4. Highlight the cells containing number values only.

9.2.5. Click on the "Insert" tab.

9.2.6. Click on "Scatter" in the "Charts" section.

9.2.7. Choose the chart-type at the top of the left column called "Scatter with only Markers" when you mouse over the choice. The chart will appear.

Title: HSA ELISA SOP

9.2.8. Right click on one of the data points in the chart and choose "Add Trendline…". The "Trendline Options" dialog box will appear.
9.2.9. Select "Linear" and check off "Display Equation on chart" and "Display R-squared value on Chart" in the "Trendline Options" dialog box.
9.2.10. Click on "Close". The line, equation and R-squared value will appear in the chart.
9.2.11. To calculate the concentration of HSA in the sample, substitute the absorbance value for "y" in the equation and solve for "x".

10. History:

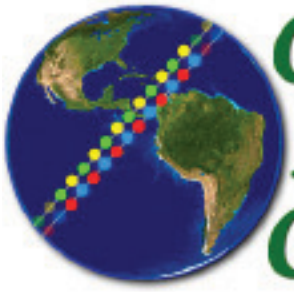
Name	Date	Amendment
Bob O'Brien	01Apr09	Initial release

Quality Control Technician (Chemistry)

REF	Key Functions & Tasks (Quality Control Chemistry Technician)
1	**Work in compliance with EH&S.**
1.a	Wear appropriate personal protective equipment.
1.b	Work in controlled environments.
1.c	Participate in emergency drills and emergency response teams.
1.d	Use routine lab safety procedures, identify unsafe conditions, and take corrective action.
1.e	Appropriately and safely access production and laboratory equipment.
1.f	Handle, label, and dispose of hazardous / biohazard materials.
1.g	Access and utilize MSDS.
1.h	Perform permitting procedures.
1.i	Carries out operations with attention to OSHA and EPA regulations, and other applicable state and federal regulations.
1.j	Participate in all company safety training and audits as required.
1.k	Assists with waste treatment operations.
1.l	Dispose of wastes .
1.m	Follows SOPs, written test procedures, safety, regulatory requirements, and approved license requirements.
1.n	Use hoods where appropriate.
1.o	Dispose of sharps properly.
2	**Work in compliance with cGMPs.**
2.a	Follow SOPs for all operations.
2.b	Records laboratory data and completes lab records as required.
2.c	Maintain equipment and instrument logbooks.
2.d	Control and receipt of raw materials.
2.e	Maintain training documentation.
2.f	Maintain equipment and methods in a validated state.
2.g	Working in controlled/classified areas (gowning, aseptic technique).
2.h	Ensure appropriate flow of personnel, equipment, and materials.
2.i	Change control for process, equipment, and documentation.
2.j	Label and apply status to equipment and materials.
2.k	Identify and report exception events and CAPA.
2.l	Review data.
2.m	Participate in change control activities.
2.n	Archive documentation/data.
3	**Clean and maintain production areas.**
3.a	Housekeeping / pest control.
3.b	Sanitize and clean of controlled spaces.
3.c	Preparation of cleaning materials and solutions
3.d	Document cleaning.
4	**Maintain effective communication.**
4.a	Maintain security and confidentiality.
4.b	Respond appropriately to internal auditors and external inspectors.
4.c	Assist in writing, reviewing, and commenting on technical documents.
4.d	Consult appropriately with internal customers.
4.e	Report result to appropriate departments.
4.f	Interact with vendors.

REF	Key Functions & Tasks (Quality Control Chemistry Technician)
5	**Receive and/or collect samples.**
5.a	Receive/collect samples (stability, in-process, water, raw materials, final product, environment, validation) per batch records or plans.
5.b	Monitor controlled equipment.
5.c	Maintain equipment logs.
5.d	Label samples appropriately.
5.e	Record sample collection and distribution (storage and chain of custody).
6	**Laboratory Work.**
6.a	Prepare reagents.
6.b	Prepare samples.
6.c	Review testing procedures
6.d	Execute testing.
6.e	Perform mathematical and statistical calculations as appropriate.
6.f	Evaluate data with regard to specification.
6.g	Perform scheduled sanitizations of hoods.
6.h	Prepare and standardize probes and ancillary instruments.
6.i	Visually inspect equipment.
6.j	Maintain equipment logs and status tags.
6.k	Complete, review and approve equipment process records.
7	**Perform Chemical and biochemical testing.**
7.a	Perform chemical and biochemical testing for identity, potency, purity, consistency, and stability.
7.b	Perform chemical and biochemical testing for raw material, water, and other environmental and validation samples.
8	**Managing Information**
8.a	Support data trending activities.
8.b	Utilize Laboratory Information Management Systems (LIMS)
8.c	Records observations, generates reports, and maintains accurate records
9	**Provide Technical and/or Validation Support**
9.a	Troubleshoots basic technical issues and investigations of OOS results, instrument malfunctions,and methodology problems.
9.b	Assist in executing validation procedures
10	**Maintain laboratory systems and equipment**
10.a	Perform preventive maintenance (PM).
10.b	Standardize laboratory instrumentation.
10.c	Identify and assist in troubleshooting instrument and equipment failures.

Appendix



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Short Protocol for Human Tissue Plasminogen Activator (tPA) Production in a Spinner Flask

By Susan Clark and Kari Britt

This protocol can be used to culture Chinese Hampster Ovary (CHO) cells in a spinner flask for Tissue Plasminogen Activator (tPA) production. This is an alternative protocol to growing the cells in a bioreactor (See: Batch Culture of Recombinant tPA Secreting CHO Cells (ATCC CRL9606) SOP). This protocol requires two spinner flasks, a 100mL and a 500mL for scaling up the production. This protocol is intended for use with the ATCC CRL 9606 CHO cell line. The cells are cultured for 5-7 days in a 100mL spinner flask and then for 5-7 days in a 500mL spinner flask.

Media Preparation

90% Ham's F-12, 10% Fetal Bovine Serum (1% Gentamycin is optional)

1. Clean, assemble and autoclave a 100mL spinner flask.
2. Aseptically combine 90mL of Ham's F-12 and 10mLof Fetal Bovine Serum. 1mL of 10mg/mL gentamycin can also be added to this solution to help prevent contamination. Antibiotic addition is highly recommended if a biological safety cabinet is not available.
3. Filter sterilize the media.
4. Aseptically transfer the media to the 100mL spinner flask.
5. Proof the media for contamination by incubating at 37°C, 5% CO_2 while spinning gently for at least 24 hours.
6. Visually observe the media for contamination. Dispose if contaminated. If not contaminated, store the media at 2-8°C until needed or proceed to inoculation.

Inoculation

1. If the media has been refrigerated, pre-warm it in the 37°C incubator until it seems like the media is approximately 37°. If the media is already warm, proceed to step 2.
2. Thaw a cryovial of ATCC CRL9606 CHO cells in a 37°C waterbath.
3. Aseptically transfer the contents of the cryovials to the 100mL spinner flask.
4. Gentle swirl the flask to mix.
5. Return the 100mL spinner flask to the incubator and incubate at 37°C, 5% CO_2 while spinning gently.
6. Immediately take a timepoint sample.

Sampling

It is recommended to take a timepoint sample every 24 hours.

1. Aseptically remove 1.5-2.0mL of culture. Take an optical density (OD) reading using a spectrophotometer. Determine the viable cell concentration using a trypan blue assay (See: Trypan Blue SOP). Take a pH reading. If a biolyzer is available measure analytes such as glucose and lactate.
2. Centrifuge the remaining sample at high speed for 5 minutes. Remove the supernatant to a clean test tube and store at 2-8°C until needed for ELISA, tPA activity assay or SDS PAGE.

Scale-Up

It is recommended to prepare and proof the media (Steps 1-5 below) for scale-up after the 100mL spinner flask has been incubating 3-4 days. After approximately 5-7 days the viable cell concentration should be approaching 1 x 10^6 cells/mL in the 100mL spinner flask. At this point scale-up to the 500mL spinner flask is recommended.

1. Clean, assemble and autoclave a 500mL spinner flask.
2. Aseptically combine 450mL of Ham's F-12 and 50mL of Fetal Bovine Serum. 5mL of 10mg/mL gentamycin can also be added to this solution.
3. Filter sterilize the media.
4. Aseptically transfer approximately 400mL of the media to the 500mL spinner flask. Set aside the excess media for use in cryopreservation.
5. Proof the media for contamination by incubating at 37°C, 5% CO_2 while spinning gently for at least 24 hours.
6. Visually observe the media for contamination. Dispose if contaminated. If not contaminated, store the media at 2-8°C until needed or proceed to inoculation (Steps 6 and 7 below).
7. If the media has been refrigerated, pre-warm it in the 37°C incubator until it seems like the media is approximately 37°. If the media is already warm, proceed to step 7.
8. Aseptically transfer the contents of the 100mL spinner flask to the media in the 500mL spinner flask.
9. Gentle swirl the flask to mix.
10. Return the 500mL spinner flask to the incubator and incubate at 37°C, 5% CO_2 while spinning gently.
11. Immediately take a timepoint sample, and continue to sample every 24hrs.

Cell Harvest and Cryopreservation

After approximately 5-7 days of incubating in the 500mL spinner flask, viable cell concentration should be approaching 1 x 10^6 cells/mL. At this point cell harvest is recommended.

1. For cryopreservation of the working cell bank a storage menstrum needs to be prepared. Combine 40mL of Ham's F-12, 5mL of Fetal Bovine Serum. Filter sterilize the media and then add 5mL of autoclaved glycerol. Alternatively, 45mL of excess media set aside during scale-up can be used. In this case, simply add 5mL of autoclaved glycerol to the media.
2. Aliquot the cell culture into 30 or 50mL centrifuge tubes. Centrifuge at 3000 x g for 10 minutes.
3. Remove the supernatant. The supernatant can be used for tPA purification (chromatography) if desired.
4. Resuspend the pellet with 1mL of storage menstrum and dispense into a 1.5mL cryovial. Store at -80°C.

Refer to the Batch Culture of Recombinant tPA Secreting CHO Cells (ATCC CRL9606) SOP for detailed step by step directions for cell harvest and cryopreservation. ELISA, SDS PAGE, tPA activity assay and chromatography standard operating procedures are included in this lab manual.

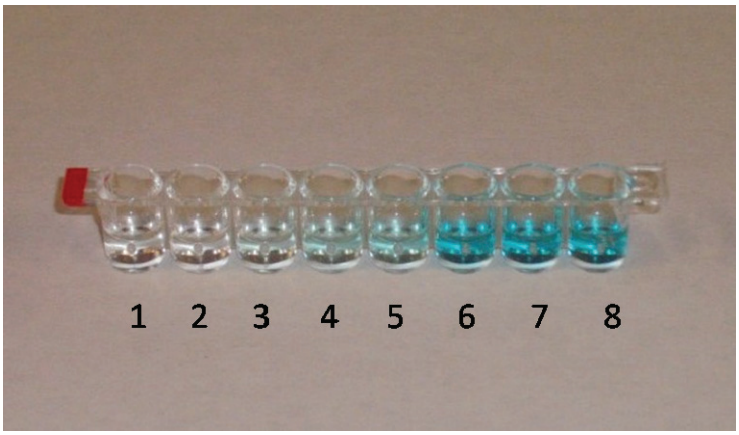

Figure 1: tPA ELISA

Well	Sample	Sample and tPA Concentration (ng/mL)
1	Blank	0
2	Standard	0
3	Standard	10
4	Standard	20
5	Standard	30
6	3rd day in 500mL flask	23.6
7	5th day in 500mL flask	26.5
8	6th day in 500mL flask	30

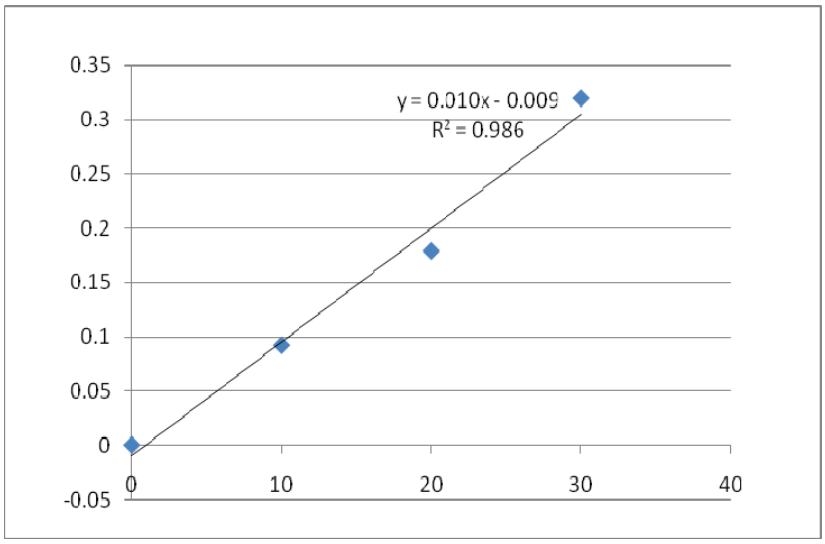

Figure 2: Standard Curve for tPA ELISA (See tPA ELISA SOP for directions to generate a standard curve).

Short Protocol for Human Serum Albumin Production in a Shake Flask

By Kari Britt and Sonia Wallman

This protocol can be used to culture *Pichia pastoris* cells in a shake flask for Human Serum Albumin (HSA) production. This is an alternative to growing the cells in a bioreactor (See: Process Controlled Fed-Batch Fermentation of Recombinant HSA Secreting *Pichia pastoris* SOP). It can also be used by the course instructor to screen *Pichia pastoris* cells for HSA production before carrying out the fed-batch fermentation in a bioreactor with the class.

Note: The timetable for this protocol has some flexibility, but is designed to be completed during a 1 week time span if desirable. For instance, if the 1L shake flask is inoculated on a Friday then by the following Friday the culture will have been exposed to methanol for 3-4 days, which should be adequate to visualize HSA production using SDS-PAGE or ELISA.

If beginning from original stab (Invitrogen: GS 1 15/HIS+/MUT-/SEC HSA):

Prepare agar plate(s) with media appropriate for growing yeast. Yeast Extract Peptone Dextrose (YEPD) media is recommended. YEPD media consists of 10g/L yeast extract, 20g/L peptone 16g/L agar and 20g/L of glucose or dextrose). Streak the desired number of plate(s) with cells from the stab. Incubate overnight at 30°C.

If beginning from master cell bank:

1. Prepare 0.1M Potassium Phosphate Medias (with and without glucose):

    1.1. Dissolve 2.6g potassium phosphate dibasic and 11.6g potassium phosphate monobasic in 1L deionized water to make 0.1M potassium phosphate buffer, pH 6. Adjust to pH 6 if necessary. Add 10g yeast extract and 20g peptone to the 0.1M potassium phosphate buffer, pH 6 to make 0.1M Potassium Phosphate Media. Place 450mL 0.1M Potassium Phosphate Media in a 1L shake flask (baffled if possible) and add 10g glucose. This is the initial glucose containing media that will be inoculated with yeast cells.

    1.2. From the remaining 0.1M Potassium Phosphate Media (without glucose) aliquot 90mL each into two autoclavable containers. One 90mL aliquot will be used to resuspend the cell culture in glucose free media and the other will be used for cryopreservation.

    1.3. Autoclave the 450mL of 0.1M Potassium Phosphate Media (with glucose) in the 1L shake flask and the two 90mL aliquots of 0.1M Potassium Phosphate Media without glucose.

1.4. Dissolve 6.7g yeast nitrogen base in 100mL deionized water to make 10X YNB. Filter sterilize this solution and aseptically add 50mL to the cooled autoclaved solution in the 1L shake flask containing 0.1M Potassium Phosphate Media (with glucose) (For a total of 500mL media in the 1L shake flask.). Aseptically add 10mL of filtered 10X YNB solution to each of the 90mL aliquots of 0.1M Potassium Phosphate Media without glucose. Refrigerate the two 100mL aliquots of 0.1M Potassium Phosphate Media, 1X YNB (without glucose) until needed.

2. Inoculate the liquid media:

2.1. Thaw a 1mL cryovial of *Pichia pastoris* cells in a 30°C water bath. Transfer the contents of the vial into the 1L shake flask and cover the flask with sterile cheese cloth. Alternatively, the shake flask can be inoculated with a colony from an agar plate grown from the stab.

Growth conditions:

Incubate the inoculated 1L shake flask at 30°C and shake at 200rpm for 3-4 days. On the 3rd or 4th day, if the culture is opaque white in appearance (OD_{600} 2-6) centrifuge the culture at 2000xG for 5 minutes. Remove the supernatant and resuspend the cells in a total of 100mL 0.1M Potassium Phosphate Media, 1X YNB without glucose. (For example if the culture was divided into 10 50mL centrifuge tubes, add 10mL of media to each tube for re-suspension.) Aseptically add the re-suspended culture to a 500mL shake flask and dispense 200µL of 100% methanol into the culture. Remove 1.5mL of culture to a microcentrifuge tube to be saved as the first time point (T_0). Return the culture to incubate at 30°C and shake at 200rpm. Add 200µL of 100% methanol to the culture every 24 hours for 3-5 days. A 2.0mL time point sample should be removed from the culture each day before adding the methanol.

Timepoints:

Remove 2.0mL from the culture at each timepoint and place in a test tube. Take an OD reading at 600nm and measure the pH. pH of 5.8-6.4 is ideal for HSA production (Barr 1992). Remove 1.5mL of the sample to a 1.5mL centrifuge tube. Centrifuge at high speed for 5 minutes. Remove the supernatant to a clean tube and store at 2-8°C until needed for SDS-PAGE and ELISA (See Figure 1 for photo of SDS-PAGE gel).

Cryopreservation:

Add 11mL sterile glycerol to the second 100mL aliquot of 0.1M Potassium Phosphate Media, 1X YNB without glucose, to make the storage media. Centrifuge the cell culture at 3000xG for 5 minutes. Re-suspend the cell pellet in a total of 10mL of storage media (For example, if the culture was divided into 10 10mL centrifuge tubes, add 1mL of storage media to each tube for re-suspension.) Aseptically dispense 1mL of re-suspended cells into each cryovials. Ideally, the cells should be stored at -80°C until needed.

Note: SDS-PAGE and ELISA standard operating procedures are included in this lab manual.

Table 1: Example of Timepoint Sample Measurments

Timepoint	pH	Optical Density
$\mathbf{T_0}$ (Day 1) Immediately following inoculation	6.01	0.00
$\mathbf{T_1}$ (Day 3)	5.92	2.08
$\mathbf{T_2}$ (Day 4)	5.85	2.51
$\mathbf{T_3}$ (Day 4) Immediately following media change and methanol addition	6.15	3.01
$\mathbf{T_4}$ (Day 5) Immediately following second addition of methanol	5.89	3.54
$\mathbf{T_5}$ (Day 6) Immediately following third addition of methanol	5.87	3.98
$\mathbf{T_6}$ (Day 7) Immediately following fourth addition of methanol	5.80	4.12

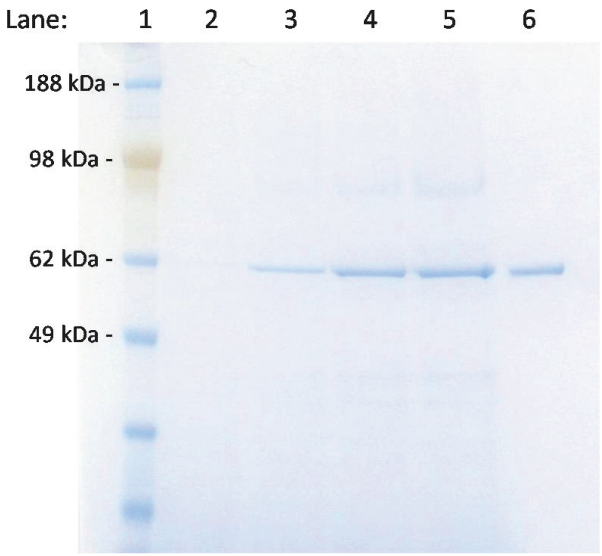

Figure 1: Photograph of SDS-PAGE Gel

Lane 1: Molecular Weight Marker (SeeBlue Plus2 Pre-Stained Standard, Invitrogen), Lane 2: $\mathbf{T_0}$ (before methanol addition), Lane 3: $\mathbf{T_4}$ (24hrs. after first methanol addition), Lane 4: $\mathbf{T_5}$ (48hrs. after first methanol addition), Lane 5: $\mathbf{T_6}$ (72hrs. after first methanol addition), Lane 6: HSA standard

Sources:

Barr, K.A., Hopkins, S.A., and Sreekrishna, K. **Protocol for Efficient Secretion of HSA Developed from *Pichia pastoris*.** Pharmaceutical Engineering. Vol. 12, No.2, 48-51. (1992).

Ohashi, R., Mochizuki, E., and Suzuki, T. **A Mini-Scale Mass Production and Separation System for Secretory Heterologous Proteins by Perfusion Culture of Recombinant *Pichia pastoris* Using a Shaken Ceramic Membrane Flask.** Journal of Bioscience and Bioengineering. Vol. 87, No. 5, 655-660. (1999).

Equipment Standard Operating Procedures

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