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Pichia pastoris - HSA
CORE PRODUCTION SYSTEM

NSF NBC² Global Biomanufacturing Curriculum

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 100x magnification
   7.4. shaking incubator (37^{o}C and 30^{o}C)
   7.5. autoclave

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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.

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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.

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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.

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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.

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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.

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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.

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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.

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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

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.

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.

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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.

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:

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**

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

Title: New Brunswick BioFlo 3000 Bioreactor SOP

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

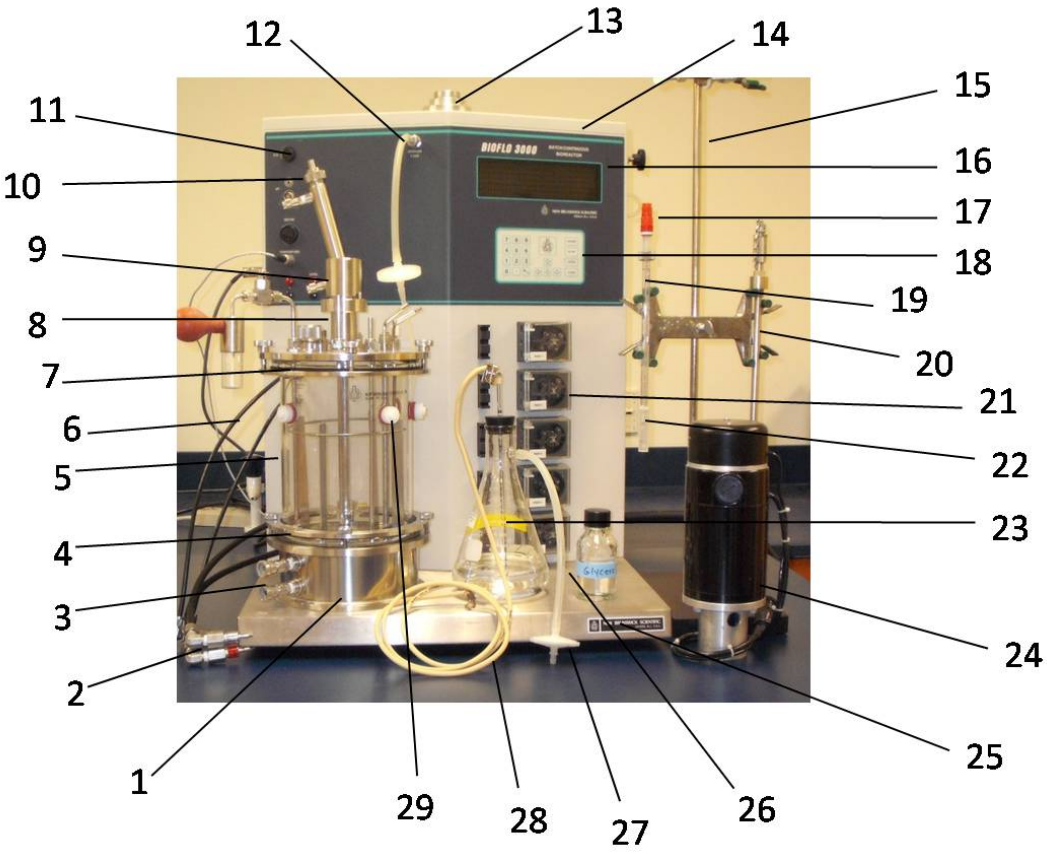

Figure 1: BioFlo 3000 Vessel and Cabinet

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

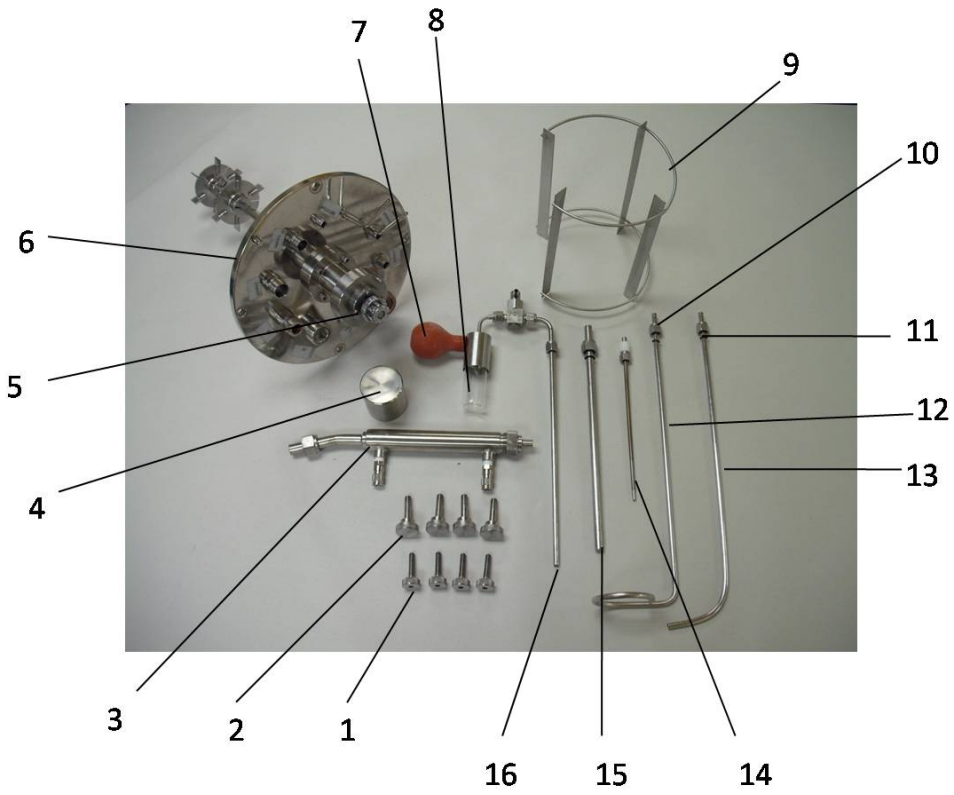

Figure 2: Vessel Parts

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

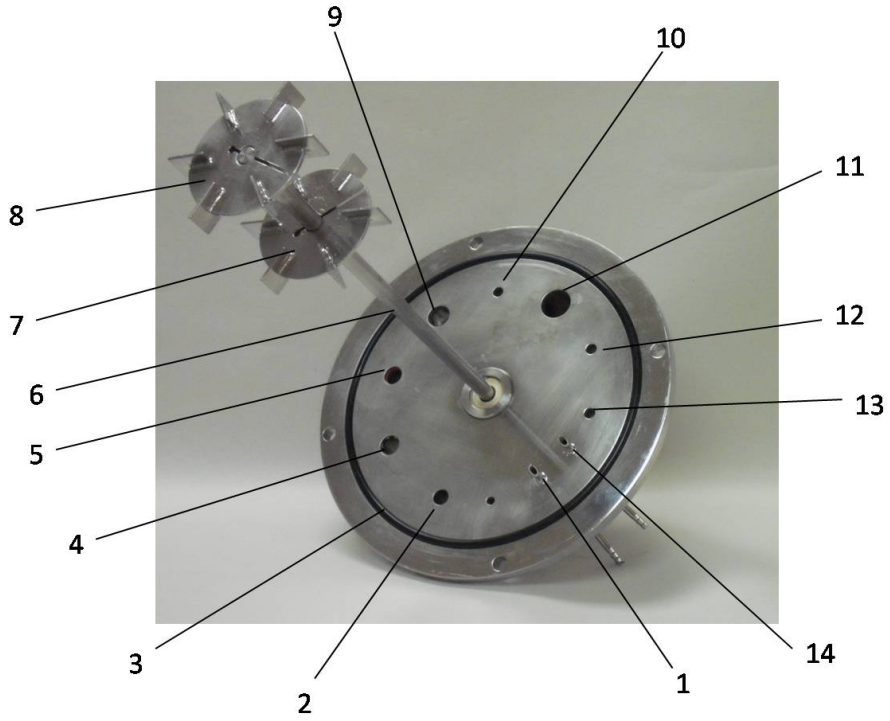

Figure 3: Headplate, Underside

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

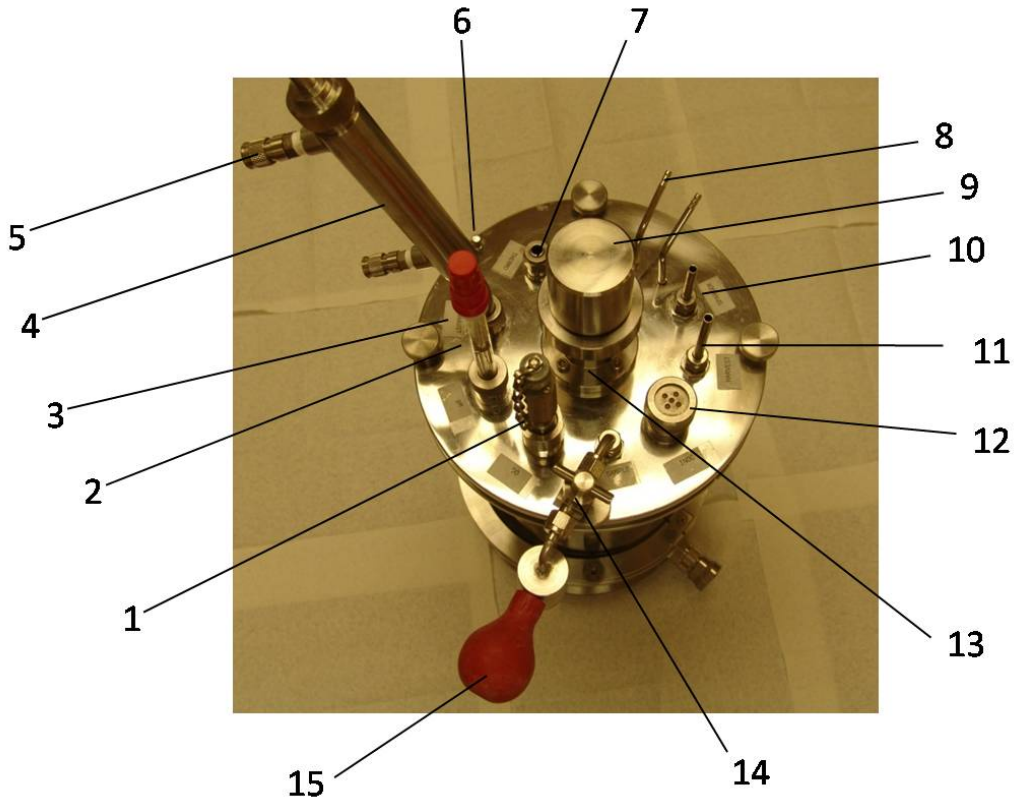

Figure 4: Assembled Headplate, Top Side

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

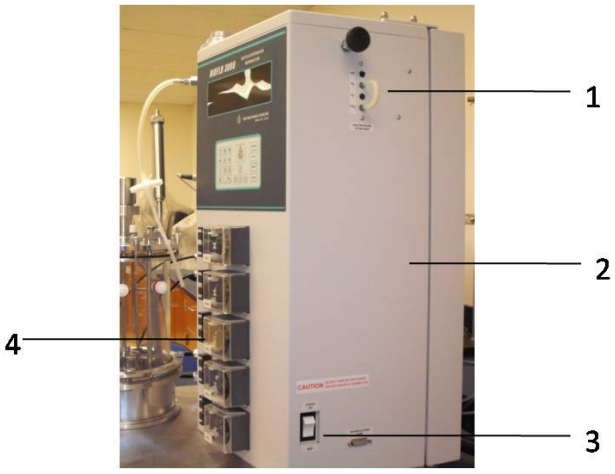

Figure 5: BioFlo 3000 Side View

1. Gas and Air Console
2. Cabinet
3. Power Switch
4. Feed Pump

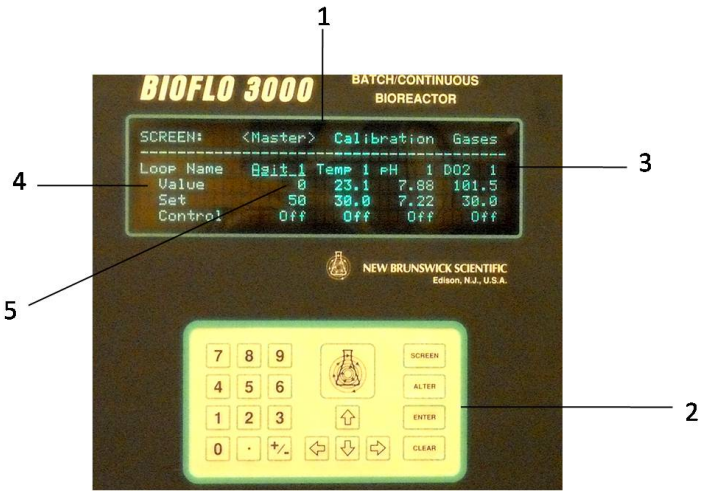

Figure 6: BioFlo 3000 Controller

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.

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

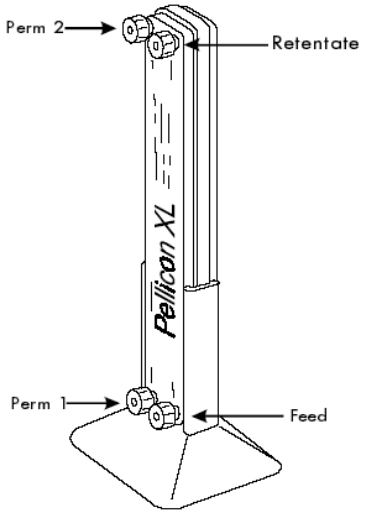

Figure 1: Millipore Pellicon XL Device
(http://www.millipore.com/userguides.nsf/docs/p60085)

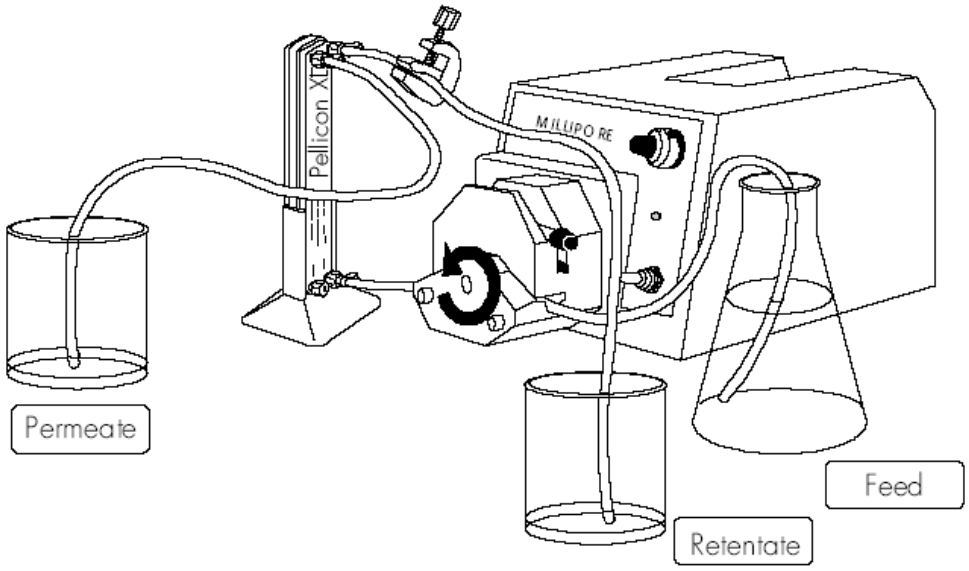

Figure 2: Set Up
(http://www.millipore.com/userguides.nsf/docs/p60085)

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.

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**

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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.

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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.

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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.

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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.

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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.

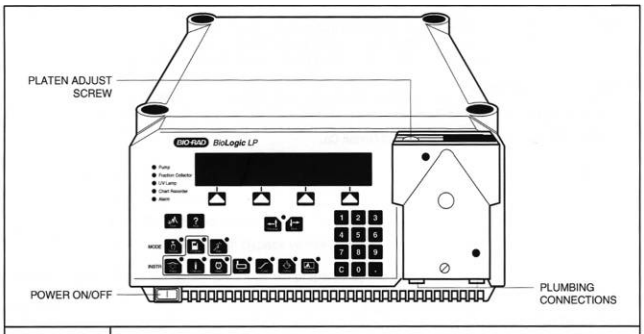
9. Attachments:

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

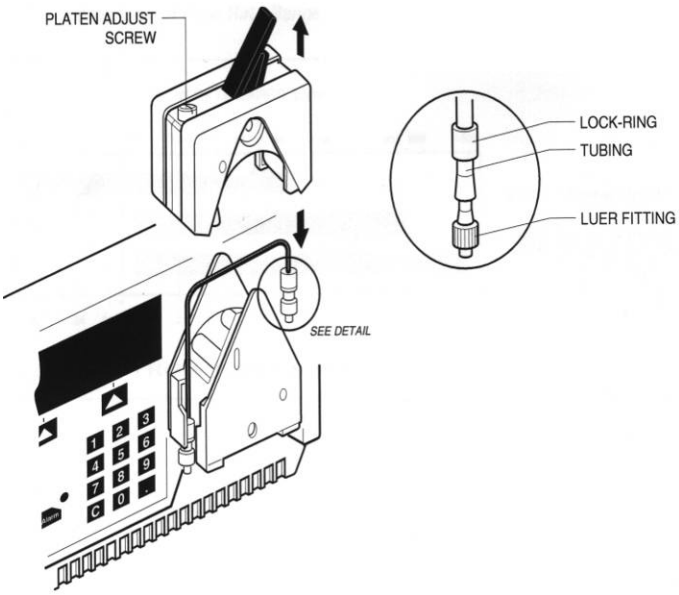


Figure 2: Controller Pump

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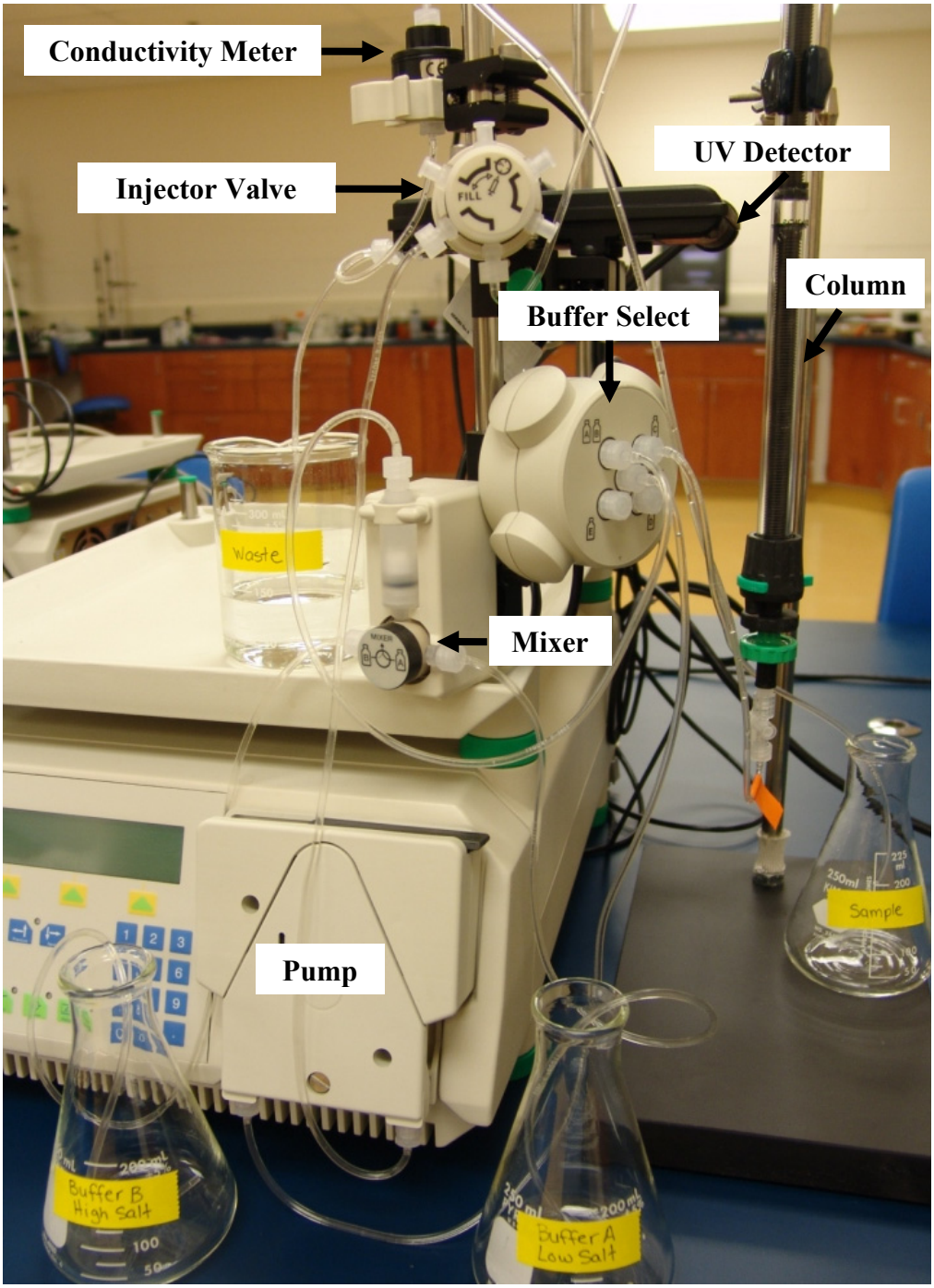

Figure 3: LP Biologic System Parts

Title: BioLogic LP Chromatography System Operating SOP

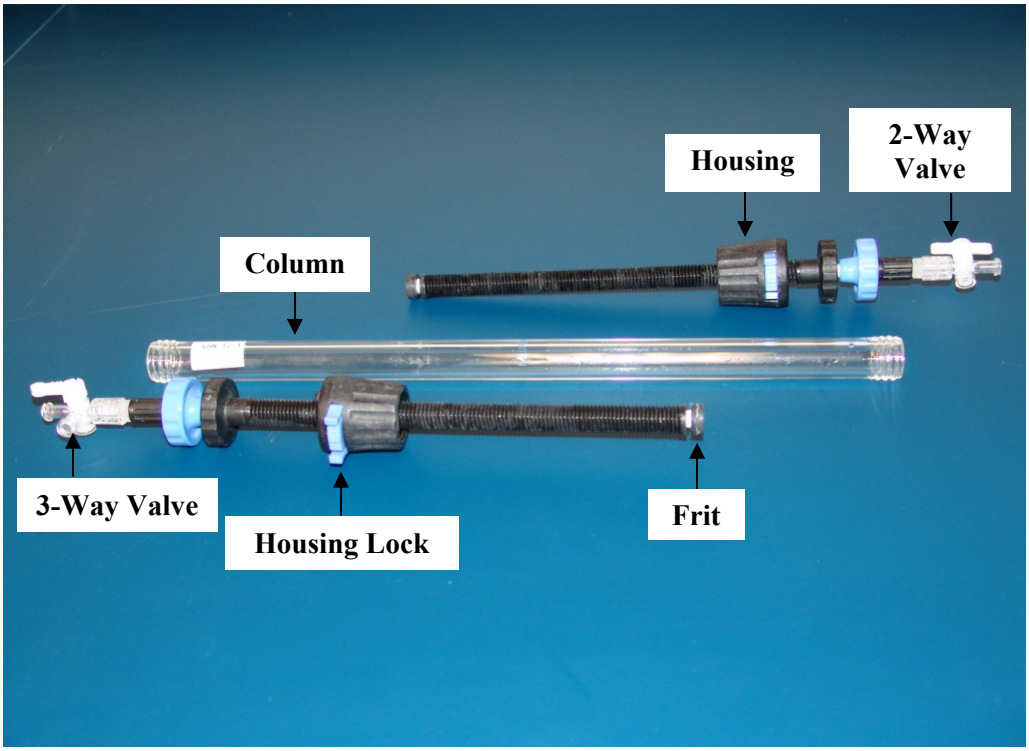

Figure 4: Column Components

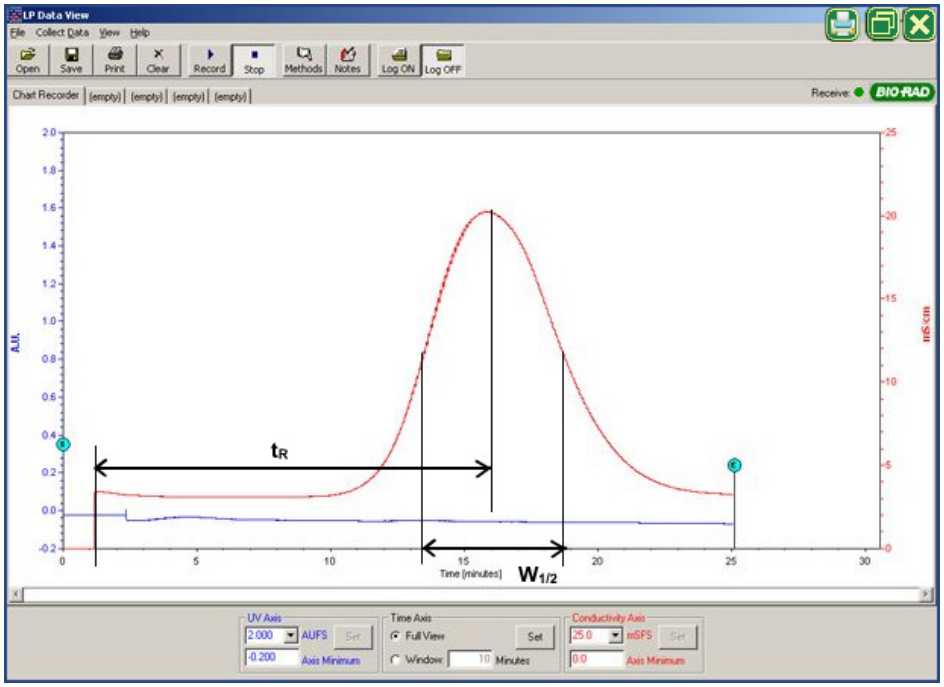

Figure 5: Chromatogram Example for Calculating HETP

Batch Record: 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.I	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.

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

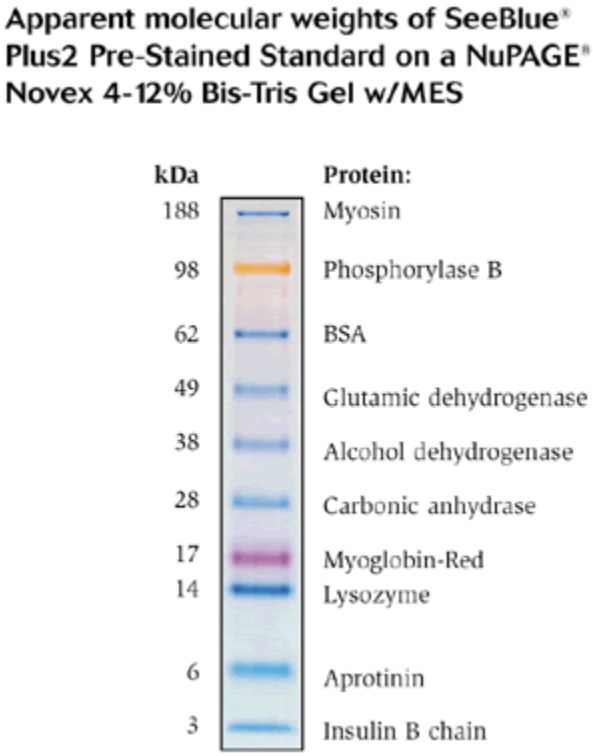

Figure 1: Molecular Weight Maker Diagram

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.

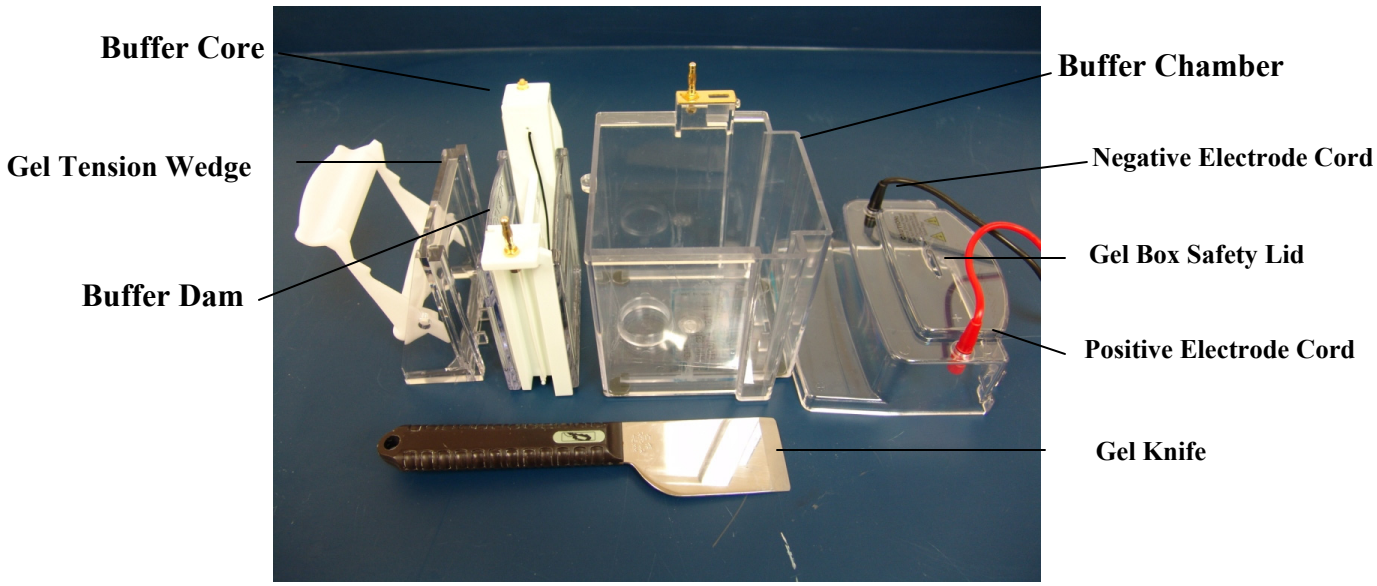

Figure 1: Gel Box Parts

Title: Xcell *SureLock* Mini-Cell Gel Box SOP

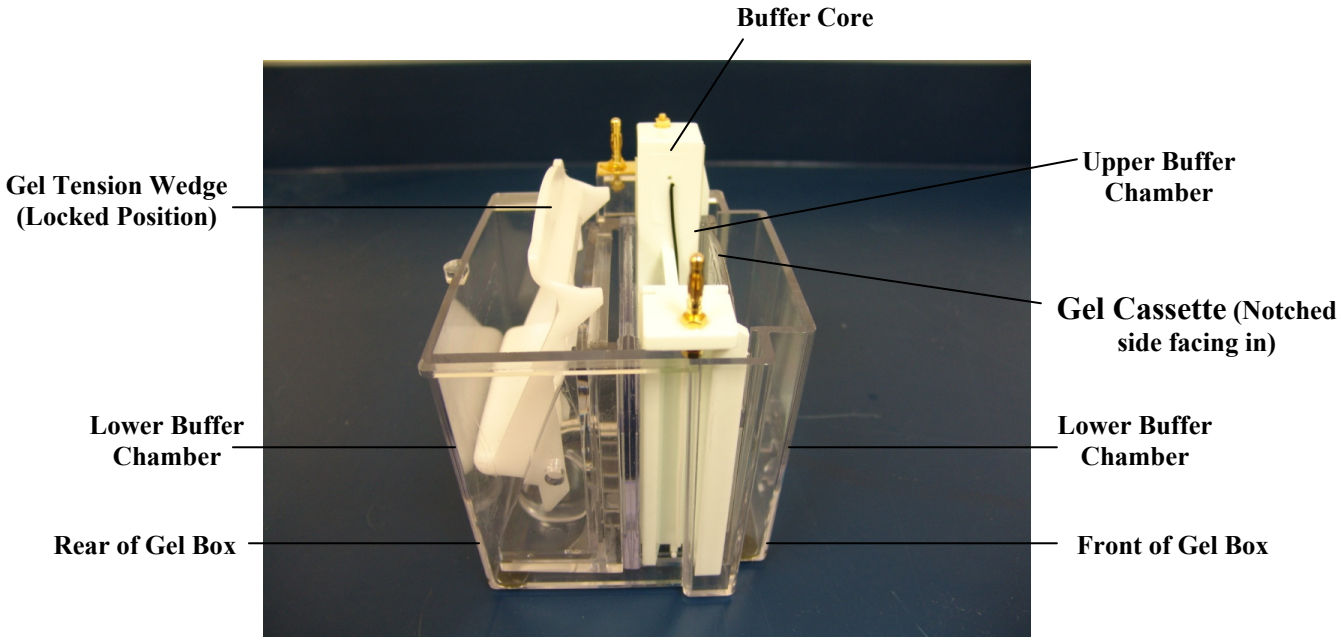

Figure 2: Assembled Gel Box Side View

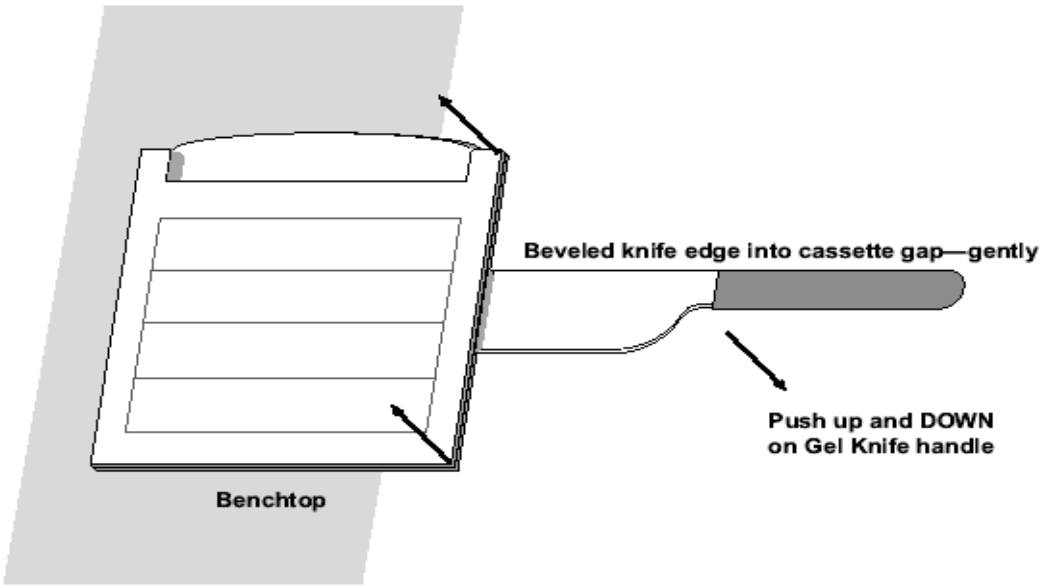

Figure 3: Opening a Gel Cassette

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:

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 12.5 ng/mL a (2mL test tube)
8. HSA 6.25 ng/mL a (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.).

8.3.2. **Plate Washing**

Title: HSA ELISA SOP

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.

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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.

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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

Title: Bio-Tek Elx808UI Automated Microplate Reader Standalone SOP

Approvals:

Preparer:___Ellery Raitt_____ Date ____28Feb05_____
Reviewer:__Deb Audino_____ Date ____28Feb05_____
Reviewer:__Sonia Wallman_____ Date ____28Feb05_____

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 components are operating properly and 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.

Title: Bio-Tek Elx808UI Automated Microplate Reader Standalone SOP

8.2.5. Press the softkey corresponding to the wavelength to be used. Press **Enter.**
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

Title: Bio-Tek Elx808UI Automated Microplate Reader Standalone SOP

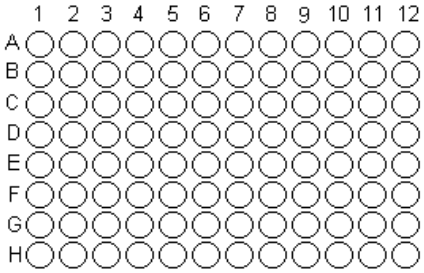

Figure 1: Microplate Map

Figure 2: Control Pad

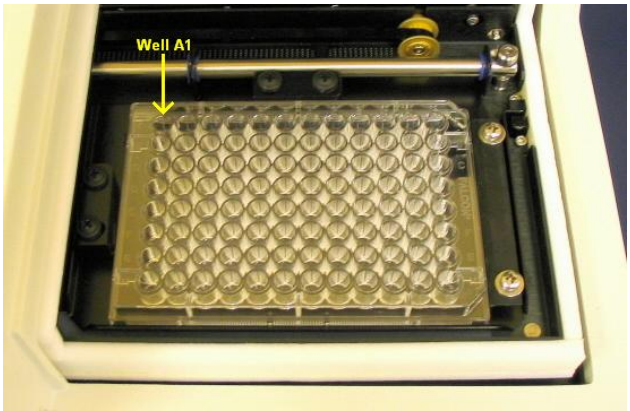

Figure 3: Loading a Microplate

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.

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

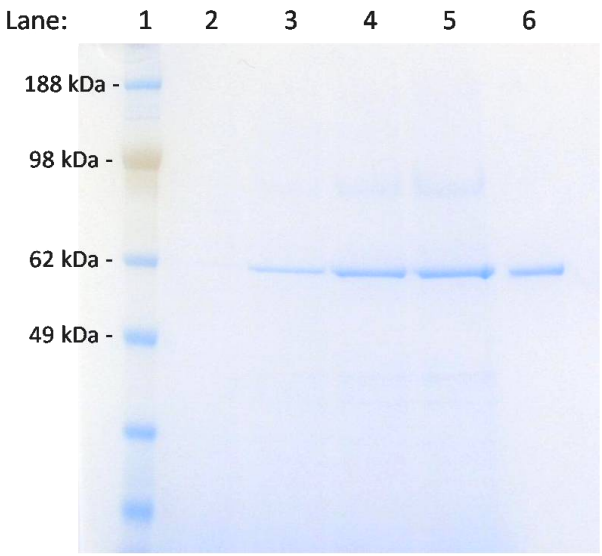

Figure 1: Photograph of SDS-PAGE Gel

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).