

Biomanufacturing Kit Reagent Sourcing, Storage and Preparation

Purpose of this Guide

This document will provide you with the following information for the Biomanufacturing kit:

- Where to order reagents & supplies for the Biomanufacturing kit
- How to store the associated reagents
- How to prepare the reagents for use in the classroom
- Which consumables and equipment are needed to run the labs in this kit

How to Use this Guide

1. Determine how much you need of supplies and reagents for the number of students/classes. Do this by using the Biomanufacturing Kit Prep Packing List that calculates how much of each reagent/consumable/equipment is needed for this kit. Use the spreadsheet to determine how much you need of each item per class (8 student groups/class).
2. Order materials needed for your labs well ahead of when you plan to use them with students.
3. When materials arrive, make sure they are stored properly. Storage information is included in this document and should also be on the packing slip.

Kit Scenario

The Biomanufacturing kit scenario provides a foundation in Biomanufacturing: Regulations (FDA, GMP, Quality Systems), Careers, Upstream Process and Downstream Process. Students work in teams for a fictional Contract Research Organization: Protein Purification Partners (PPP). All teams are made of up new hires to PPP who must be trained before beginning their first assignment - purification of Red Fluorescent Protein (RFP) or Green Fluorescent Protein (GFP).

Module 1, Lessons 1-5:

Module 1, Lessons 1-5 Ordering Information

The supplies needed for Module 1 (New Employee Onboarding and Training) are basic office supplies such as:

- Paper of various sizes
- Sticky notes
- Pens and Pencils
- Tape

- Folders
- Printed copies of various documents

Module 2, Lesson 1: Introduction to aseptic technique

Module 2, Lesson 1 Ordering Information

Name	Source	Cat or Model #	Storage	Requires prep before class?*
60 x 15mm sterile petri dishes – for small LB agar plates	Fisher Scientific – or other scientific supply company	Falcon #353002	Room temperature	
LB liquid media - made from LB media powder	LB powder – Any scientific supply company	Fisher Scientific BP 1426-500 (500g)	Powder is stored at room temp. The sterile liquid LB media is stored at room temp. if sterile and unopened. Store in the refrigerator once opened.	Need to make liquid LB prior to class.
LB Agar for making LB Bacterial plates	Any Scientific supply Co (LB Agar)	Fisher Scientific BP1425-500 (500g)	LB Agar powder stored at room temperature. LB Agar solution is poured into petri dishes which are then stored in the refrigerator	Need to pour plates ahead of time. Dry and pre-warm plates in 37 °C incubator prior to use
Sterile Plastic Bacterial Spreaders	Any scientific supply company	Thomas Scientific 1213M66	Room temperature	
10% bleach solution	Make in-house		Room Temperature	

* See prep directions in following section

Module 2, Lesson 1 Preparation Directions

The following reagents need to be prepped before Module 2, Lesson 1:

- 1) **Liquid LB Media** – See **Appendix A**
- 2) **LB Agar Plates** – See **Appendix B**
- 3) **10% Bleach**
 - a. Mix 90mL of di water and 10mL of bleach
 - b. Store at room temperature for up to 4 weeks

Module 2, Lesson 2: Bacterial Inoculation

Module 2, Lesson 2 Ordering Information

Name	Source	Cat or Model #	Storage	Requires prep before class?
LB/Amp liquid media - made from LB media powder	LB powder – Any scientific supply company	Fisher Scientific BP 1426-500 (500g)	Powder is stored at room temp. The sterile liquid LB media is stored at room temp. if sterile and unopened. Store in the refrigerator once opened.	Need to make liquid LB/Amp prior to class.
Ampicillin	Sigma Aldrich Ampicillin Sodium Salt	A9518	1,000X stock stored at -20°C	Need to make 1,000X stock prior to class
Sterile inoculating loop	Fisher Scientific or any scientific supply company	Fisher Sci #22363605, 250/case	Room temperature	
14mL Falcon tubes polystyrene round-bottom tubes, 17x100mm style	Fisher Scientific or any scientific supply company	Falcon, Ref 352057	Room temperature	
10% bleach solution	Make in-house		Room Temperature	

* See prep directions in following section

Module 2, Lesson 2 Preparation Directions

The following reagents need to be prepped before Module 2, Lesson 1:

- 1) **Liquid LB/Amp Media – See Appendix C**
- 2) **A bacterial plate containing RFP+ or GFP+ bacterial colonies** – This can be made by using the ABE labs or by using the pGLO BIORAD kit.
- 3) **10% Bleach**
 - a. Mix 90mL of di water and 10mL of bleach
 - b. Store at room temperature for up to 4 weeks

Module 2, Lesson 3: Bacterial Culture Scale-Up

Module 2, Lesson 3 Ordering Information

Name	Source	Cat or Model #	Storage	Requires prep before class?
LB/Amp liquid media - made from LB media powder	LB powder – Any scientific supply company	Fisher Scientific BP 1426-500 (500g)	Powder is stored at room temp. The sterile liquid LB media is stored at room temp. if sterile and unopened. Store in the refrigerator once opened.	Need to make liquid LB prior to class.
Ampicillin sodium salt	Sigma-Aldrich	A9518	1000X stock stored in -20 °C freezer	Need to make 1000X stock. Thaw on ice prior to adding to LB Agar
125mL sterile baffled Erlenmeyer flasks with lids	Any scientific supply company like ThermoFisher etc		Room temperature after autoclaving	Autoclave flasks with lids before class
50mL sterile plastic test tubes	Any scientific supply company		Room temperature	
Spectrophotometer cuvettes	Ecuvettes or any scientific supply company	10mm optical path length, 3.5mL, 285-750nm, disposable plastic cuvettes	Room temperature	
10% bleach solution	Make in-house		Room Temperature	

** See prep directions in following section*

Module 2, Lesson 3 Preparation Directions

The following reagents need to be prepped before Module 2, Lesson 3:

- 1) Liquid LB/Amp Media – See Appendix B**
- 2) 10% Bleach**
 - a. Mix 90mL of di water and 10mL of bleach
 - b. Store at room temperature for up to 4 weeks

Module 2, Lesson 4: Induction of RFP or GFP Production

Module 2, Lesson 4 Ordering Information

Name	Source	Cat or Model #	Storage	Requires prep before class?
L- (+) – Arabinose – powder	Sigma-Aldrich	A91906-100G-A	Room Temperature Stable for one month max.	Need to make sterile 50X stock. Or purchase 20% solution (see next row)
L- (+) – Arabinose – solution	Teknova	A2010 20% solution Sterile	Room temperature	

* See prep directions in following section

Module 2, Lesson 4 Preparation Directions

The following reagents need to be prepped before Module 2, Lesson 4:

1) 50X Arabinose Stock –

- Dissolve 3g Arabinose in 20mL distilled water for a 50X (15%) stock.
- Sterile filter over a 0.22um filter.
- Store at room temperature for up to one month.

NOTE: A sterile 20% solution of Arabinose can be purchased and used instead.

Module 2, Lesson 5: Bacterial Harvest and Determination of Bacterial Yield

Module 2, Lesson 5 Ordering Information

Name	Source	Cat or Model #	Storage	Requires prep before class?
LB Agar for making LB/Amp Bacterial plates	Any Scientific supply Co (LB Agar)	Fisher Scientific BP1425-500 (500g)	LB Agar powder stored at room temperature. LB Agar solution is poured into petri dishes which are then stored in the refrigerator	Need to pour plates ahead of time. Dry and pre-warm plates in 37 °C incubator prior to use

100 x 15mm petri dishes – for med LB Agar/Amp plates	Fisher Scientific – or other scientific supply company	Falcon #351029	Room Temperature	
Ampicillin sodium salt	Sigma-Aldrich	A9518	1000X stock stored in -20 °C freezer	Need to make 1000X stock. Thaw on ice prior to adding to LB Agar
Sterile Plastic Bacterial Spreaders	Any scientific supply company	Thomas Scientific 1213M66	Room temperature	
1.5mL microfuge tubes	Any scientific supply company		Room Temperature	
10% bleach solution	Make in-house		Room Temperature	

* See prep directions in following section

Module 2, Lesson 5 Preparation Directions

The following reagents need to be prepped before Module 2, Lesson 5:

- 1) **Liquid LB/Amp Agar Plates – See Appendix B**
- 2) **10% Bleach**
 - a. Mix 90mL of di water and 10mL of bleach
 - b. Store at room temperature for up to 4 weeks

Module 3, Lesson 1: Bacterial Lysate

Module 3, Lesson 1 Ordering Information

Name	Source	Cat or Model #	Storage	Requires prep before class?
Lysis Buffer (BPER Solution)	Thermo Scientific	78243	Room temperature	
TE Buffer – (10mM Tris, 1mM EDTA)	Make in-house or purchase from Sigma Aldrich or other company	8910-OP (Sigma)	Room temperature	
1.5mL microfuge tubes	Any scientific supply company		Room temperature	
10% bleach solution	Make in-house		Room Temperature	

* See prep directions in following section

Module 3, Lesson 1 Preparation Directions

The following reagents need to be prepped before Module 3, Lesson 1:

- 1) **Liquid LB/Amp Agar Plates – See Appendix A**
- 2) **10% Bleach**
 - a. Mix 90mL of di water and 10mL of bleach
 - b. Store at room temperature for up to 4 weeks

Module 3, Lesson 2: Column Purification

Module 3, Lesson 2 Ordering Information

** See prep directions in following section*

Name	Source	Cat or Model #	Storage	Requires prep before class?
HIC Chromatography Columns and caps	BioRad	1660413EDU	Room temperature	
Column Binding Buffer – 4M (NH ₄) ₂ SO ₄ in TE, pH8	Ammonium Sulfate – VWR	BDH9216-2.5kg	Room temperature	Yes
Column Equilibration Buffer – 2M (NH ₄) ₂ SO ₄	Made from column binding buffer		Room temperature	Yes
Column Wash Buffer – 1.3 M (NH ₄) ₂ SO ₄	Made from column binding buffer		Room temperature	Yes
Elution Buffer (TE) pH 8 – (10mM Tris, 1mM EDTA)	Made in-house or purchase from Sigma Aldrich or other company	8910-OP (Sigma)	Room temperature	Yes

** See prep directions in following section*

Module 3, Lesson 2 Preparation Directions

The following reagents need to be prepped before Module 3, Lesson 2:

- 1) **Column Binding Buffer (4M (NH₄)₂SO₄ in TE, pH8) – See Appendix C**
- 2) **Column Equilibration Buffer (2M (NH₄)₂SO₄ in TE, pH8) –**
 - a. Mix equal volumes of distilled water and column binding buffer
 - b. Store at room temperature
- 3) **Column Wash Buffer (1.3M (NH₄)₂SO₄ in TE, pH8) –**
 - a. Mix one volume of Column Binding Buffer with 2 volumes of di water
 - b. Store at room temperature.

NOTE: A column buffer pack can be purchased from BioRad (1660016EDU)

4) Elution Buffer (TE pH 8 = 10mM Tris, 1mM EDTA) –

- a. Make in-house: <https://gatescientific.com/technique-geeks-blog/f/recipes-for-te-tris-edta?srsId=AfmBOorZzTrLcBQhKajXloCPW6BiHJFVtw05AQSDStrkGxlc7CcNLeZ>
- b. Or – purchase pre-made (see information above)

5) Purification columns – purchase from BioRad (1660413EDU)

Module 3, Lesson 3: Protein Concentration Standard Curve

Module 3, Lesson 3 Ordering Information

** See prep directions in following section*

Name	Source	Cat or Model #	Storage	Requires prep before class?
Coomassie Protein Assay Reagent + Albumin standard	Thermo Scientific	1856209	Refrigerator	
Spectrophotometer cuvettes	Ecuvettes or any scientific supply company	10mm optical path length, 3.5mL, 285-750nm, disposable plastic cuvettes	Room temperature	Yes
Elution Buffer (TE) pH 8 – (10mM Tris, 1mM EDTA)	Made in-house or purchase from Sigma Aldrich or other company	8910-OP (Sigma)	Room temperature	Yes
0.5mL microfuge tubes	Any scientific supply company		Room temperature	
5mL Falcon Tubes – BD Falcon 5mL polypropylene round bottom tubes, 12 x 75mm style	Any scientific supply company	#352063	Room temperature	

** See prep directions in following section*

Module 3, Lesson 3 Preparation Directions

The following reagents need to be prepped before Module 3, Lesson 3:

1) Elution Buffer (TE pH 8 = 10mM Tris, 1mM EDTA) –

- a. Make in-house: <https://gatescientific.com/technique-geeks-blog/f/recipes-for-te-tris-edta?srsId=AfmBOorZzTrLcBQhKajXloCPW6BiHJFVtw05AQSDStrkGxlc7CcNLeZ>
- b. Or – purchase pre-made (see information above)

Module 3, Lesson 4: Measurement of RFP or GFP Protein Concentration

Module 3, Lesson 4 Ordering Information

Name	Source	Cat or Model #	Storage	Requires prep before class?
Coomassie Protein Assay Reagent + Albumin standard	Thermo Scientific	1856209	Refrigerator	
Spectrophotometer cuvettes	Ecuvettes or any scientific supply company	10mm optical path length, 3.5mL, 285-750nm, disposable plastic cuvettes	Room temperature	Yes
Elution Buffer (TE) pH – (10mM Tris, 1mM EDTA)	Made in-house or purchase from Sigma Aldrich or other company	8910-OP (Sigma)	Room temperature	Yes
0.5mL microfuge tubes	Any scientific supply company		Room temperature	
5mL Falcon Tubes – BD Falcon 5mL polypropylene round bottom tubes, 12 x 75mm style	Any scientific supply company	#352063	Room temperature	

* See prep directions in following section

Module 3, Lesson 4 Preparation Directions

The following reagents need to be prepped before Module 3, Lesson 4:

1) Elution Buffer (TE pH 8 = 10mM Tris, 1mM EDTA) –

- a. Make in-house: <https://gatescientific.com/technique-geeks-blog/f/recipes-for-te-tris-edta?srsId=AfmBOorZzTrLcBQhKajXloCPW6BiHJFVtw05AQSDStrkGxlc7CcNLeZ>

- b. Or – purchase pre-made (see information above)

Equipment Required for Biomanufacturing Kit

Name	Source	Example Source Cat or Model #
Micropipette tips P20 P200 P1000	Any scientific supply company	
Micropipettes P20 P200 P1000	Multiple sources	Gilson, Rainin
Mini Microfuges	Multiple sources	
Tube racks	Multiple sources	
Larger tabletop microcentrifuge	Multiple sources	
Column Holders	Ring Stands and clamps or other racks	VWR, Sigma
Heat block	Multiple sources	
Portable bacterial plate incubator	Multiple sources	
Portable shaker/incubator	Multiple sources	BenchMark Incu-Shaker Mini
Spectrophotometer that can measure OD 600 and 595	Multiple sources	
Autoclave		
Ice buckets	Multiple sources	

Appendix A

LB Liquid Media

1. The liquid LB media is prepared by adding 2.5 g LB media powder (Fisher Scientific #BP1426) per 100mL distilled water. Prepare the desired amount in an autoclavable glass bottle. Do not fill the bottle all the way to the top.
2. Cap the bottle and mix vigorously by swirling. Small chunks of powder will remain but they will disappear during the autoclaving process.

3. Loosen the bottle cap and apply autoclave tape.
4. Autoclave on 'liquid' setting for 20 minutes to sterilize the solution.
5. Allow the autoclave to cool a bit before opening the door. Vent the steam before attempting to open the door all the way and remove the bottles.
6. Wear the appropriate gloves for removing very hot items.
7. Store at room temperature until opened. After opening, store in the refrigerator.

LB Liquid Media + Amp

1. Make and sterilize LB media as described above.
2. Thaw one tube of sterile 1,000X Ampicillin stock (100 mg/mL)
3. Add the appropriate amount of ampicillin to the volume of LB media you need.
4. Put the cap on the bottle or tube and swirl to mix.
5. Store in the refrigerator for up to 4 weeks.

Ampicillin 1,000 X Stock (100 mg/mL)

1. Mix 2 g Ampicillin sodium salt powder into 20 mL of MilliQ (or DI) water.
2. Vortex until powder is thoroughly dissolved.
3. Sterilize using 50 mL syringe with syringe filter into 50 mL conical tube.
4. Aliquot 0.5-1 mL of Amp solution per 1.5 mL tube and store at -20 °C.

Note: Filtering and aliquoting are performed in a sterile tissue culture hood.

Appendix B

LB Agar Plates

1. The LB Agar is prepared by adding 4g LB Agar (Fisher Scientific #BP1425) per 100mL distilled water. Prepare the desired amount in an autoclavable glass bottle. Do not fill the bottom all the way to the top.

2. Add a magnetic stir bar to the bottle.
3. Loosen the bottle cap and apply autoclave tape.
4. Autoclave on 'liquid' setting for 20 minutes to melt the agar and sterilize the solution.
5. Allow the autoclave to cool a bit before opening the door. Vent the steam before attempting to remove the bottles.
6. Wear the appropriate gloves for removing very hot items.
7. Remove the bottles and place on stirrers.
8. Stir the solution while it cools in order to keep the melted agar (which is still heavier than water) in homogeneous suspension.
9. While the LB agar solution is stirring and cooling, label your plates.

For LB: using a sharpie pen, draw two black lines down the edge of the plates, marking both the lid and bottom of the plate.

LB/Amp: mark with two black lines and one red line.
10. Once the bottles have cooled enough to be handled (approx. 60 deg C), pour the plates.
11. Do not allow the bottles to cool too much, or the agar will begin to harden in the bottle.
12. Lay out your plates and remove the lids.
13. Pour enough LB agar solution in the bottom of each plate to cover it - about 20-30mL per 100mm plate.
14. Put the lids on the plates and let them harden/cool.
15. If you are not using the plates right away, stack them, bag them and store them in the refrigerator upside down (so that condensation does not drip back onto the plate).

LB/Amp agar plates

1. Prepare plates as above.
2. Thaw some sterile ampicillin on ice. The Amp stock is 100mg/mL, which is 1,000X.

3. When agar has cooled enough for the bottle to be handled, add Ampicillin (1uL Amp/mL of LB agar solution).
4. Allow the solution to stir for a few minutes.
5. Pour plates as described above. Store plates as described above.

Ampicillin 1,000 X Stock (100 mg/mL)

1. Mix 2 g Ampicillin sodium salt powder into 20 mL of MilliQ (or DI) water.
2. Vortex until powder is thoroughly dissolved.
3. Sterilize using 50 mL syringe with syringe filter into 50 mL conical tube.
4. Aliquot 0.5-1 mL of Amp solution per 1.5 mL tube and store at -20 °C.

Note: Filtering and aliquoting are performed in a sterile tissue culture hood.

I like to pour all the LB agar plates first. Then I add the appropriate amount of Amp to the remaining LB agar and stir to get it evenly mixed in. Next, I pour the LB/Amp agar plates. After that I add the appropriate amount of Arabinose powder to the remaining LB/Amp agar and stir a couple of minutes to get it into solution. Lastly, I pour the LB/Amp/Ara plates.

Be sure to mark the petri plates first with the appropriate lines down the side from top to bottom of the plate.

- Two black lines = LB agar
- Two black lines + one red line = LB/Amp agar
- Two black lines + one red line + one blue line = LB/Amp/Ara

Appendix C

Column Binding Buffer – 4M (NH₄)₂SO₄

1. To prepare 1L: Add 258.5 g (NH₄)₂SO₄ to 750mL TE (10mM Tris, 1mM EDTA).
2. Mix using a magnetic stirrer.
3. Bring to 1L with TE.
4. Store at room temperature.

