

Biomanufacturing Kit Supplies/Reagents List

Supplier and ordering information are supplied, but many of these reagents and consumables can be purchased from multiple different companies.

Module 1

This module requires mostly office supplies such as paper, pens, tape etc.

The GMP game board and cards should be printed and laminated.

Module 2

- Sterile LB (liquid media for growing bacteria) – LB broth powder – Miller, 500g, BBP1426-500
 - Make up the media in a glass bottle, according to manufacturer's instruction. Autoclave to sterilize.
- Sterile LB/Amp – LB broth plus ampicillin
 - Make sterile LB broth as described above. Cool broth to and add Ampicillin (from a 1000 X stock) to the broth.
- 1000 X Ampicillin (100mg/mL)
 - Ampicillin Sodium Salt (powder), Sigma Aldrich #A9518
Dissolve the appropriate amount of Ampicillin Sodium Salt in distilled water to make a 100mg/mL solution. Filter sterilize over a 0.2um filter. Aliquot and store at -20 deg C. You can purchase the Amp already in solution at 100mg/mL – Sigma Aldrich #A5354
- Small LB agar plates
 - LB Agar powder – Miller, 500g, 1425-500. Make in a glass bottle according to manufacturer's instructions. Add a magnetic stir bar to the bottle. Autoclave to sterilize. Put on stir plate and stir to keep the agar solution homogeneously mixed while it cools. Once it is cool enough to safely handle the bottle, dispense 6mL/60x15mm petri dish.
 - 60 x 15mm sterile petri dishes – Falcon, Ref 353002
- Medium LB/Amp agar plates
 - 100 x 15mm petri dishes – Falcon, Ref# 351029 Make as above but add Ampicillin (from 1000X stock) to the LB agar once it has cooled enough to make it possible to pick up the bottle. Pour 10-20mL/plate.
- Sterile inoculating loop – Fisher Scientific, 22363605, 250/case
- Sterile L-shaped bacterial spreaders – Fisher Scientific, 14-665-230, 10/bag
- 14mL Falcon tubes – polystyrene round-bottom tubes, 17x100mm style, Falcon, Ref 352057
- 50X sterile, liquid Arabinose stock
 - L- (+) – Arabinose – Sigma/Aldrich, A91906-100G-A
Dissolve 3g Arabinose in 20mL distilled water for a 50X stock. Sterile filter.
- Spectrophotometer cuvettes
 - Purchase the cuvettes that work with the spectrophotometer you will be using. The cuvettes we use with the specs we loan out are: 10mm optical path length, 3.5mL, 285-

750nm, disposable plastic cuvettes from Ecuvettes: <https://ecuvettes.com/product-category/products/cuvettes/all-cuvettes-and-cells/plastic-disposable-cuvettes/>

- Sterile 50mL plastic test tubes – available from many scientific supply vendors
- P200 micropipette tips – available from many scientific supply vendors
- P1000 micropipette tips – available from many scientific supply vendors
- 1.5mL microfuge tubes – available from many scientific supply vendors

Module 3

- Lysis Buffer – B PER Reagent, Thermo Scientific, #78243, 165mL
- Column Binding Buffer – 4M (NH₄)₂SO₄ in TE, pH 8
 - Ammonium Sulfate – VWR, BDH9216-2.5kg
- Column Equilibration Buffer – 2M (NH₄)₂SO₄
 - Dilute Column Binding Buffer (4M) 1:1 with distilled water (ie one part 4M (NH₄)₂SO₄ + one part water)
- Column Wash Buffer – 1.3 M (NH₄)₂SO₄
 - Dilute Column Binding Buffer (4M) 1:2 with distilled water (ie one part 4M (NH₄)₂SO₄ + 2 parts water)
- Elution Buffer (TE) – (10mM Tris, 1mM EDTA) – This can be purchased or made in the lab.
- Column Supplies

You can purchase columns and solutions from BIO RAD:

Kit containing 8 columns and all solutions: <https://www.bio-rad.com/en-us/product/green-fluorescent-protein-chromatography-kit?ID=7f20553c-73f8-498a-b0b1-75d985308bf8>

8 columns only: <https://www.bio-rad.com/en-us/sku/1660413EDU-hic-chromatography-columns-caps?ID=1660413EDU>

These columns are part of a kit to use column chromatography to purify Green Fluorescent Protein (GFP). They use the same resin that I use to make columns to purify RFP.

Or you can order individual components and make your own columns:

- Column Resin – Micro Prep Methyl HIC (hydrophobic interaction chromatography) Media, BioRad, #156-0080, 100mL
- Frits: part #GP-208, size 0.375X0.080 “, 20micron, minimum order of #2000 for \$142.76
GenPore - 1136 Morgantown Road, PO Box 380, Reading, PA 19607 USA
610-374-5171
- Barrels - 3cc manual syringe **barrels** luer lock, pk 50/\$12.71 - part 403LL-1
- luer lock **tip cap** seal, pk 50/ \$7.44 part 7015LLWPK
- 3cc snap on **end cap**, pk 50/ \$18.32 - part 703000CRPK
Barrels, Tip caps, End caps - Adhesive Dispensing LTD <https://www.adhesivedispensing.co.uk/>

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- Stopcocks: You want 2-way luer lock, do not need to be sterile, best to get ones without the extra luer lock over the male end (where the students would collect the RFP)
Unfortunately Cole Parmer no longer carries these but here are some substitutes:

Flinn Scientific: Item # AP9159, \$5.24 each

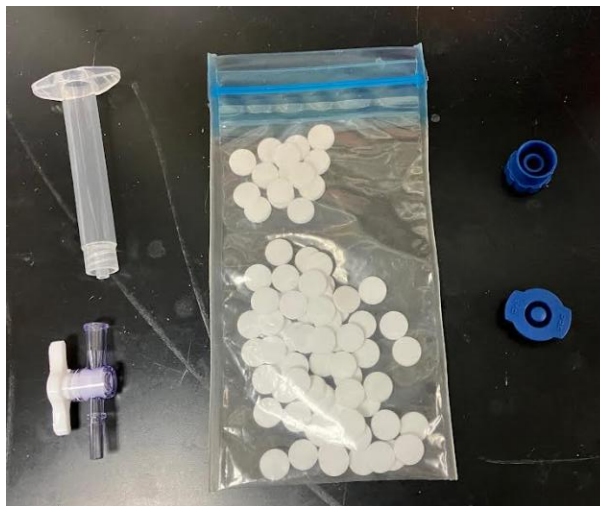
Amazon: https://www.amazon.com/Kimble-Chase-420163-0001-One-Way-Stopcock/dp/B01MR0F5KX/ref=sr_1_1?keywords=two+way+stop+cock+luer+lock&qid=1680232415&sr=8-1
\$97.71/pk 10

Amazon: https://www.amazon.com/OZCZKZZ-Stopcock-Connections-Sterile-Individual/dp/B0BTRP7JMT/ref=asc_df_B0BTRP7JMT/?tag=hyprod-20&linkCode=df0&hvadid=647246591746&hvpos=&hvnetw=g&hvrnd=15707320770076498750&hvpone=&hvptwo=&hvqmt=&hvdev=c&hvdvcmdl=&hvlocint=&hvlocphy=9031183&hvtargid=pla-1991182200154&psc=1&gclid=CjwKCAjw5pShBhB_EiwAvmnNVyXKNw-GfCrCJaS0n-04AxK17l-kcIYVwbDnGov8WGMEOffNQOPxYRoCTF8QAvD_BwE
these ones have an extra piece on the end that I cut off, \$9.90/6

BioRad: item # 7328102, 10/ \$78

Column Barrel

Stopcock

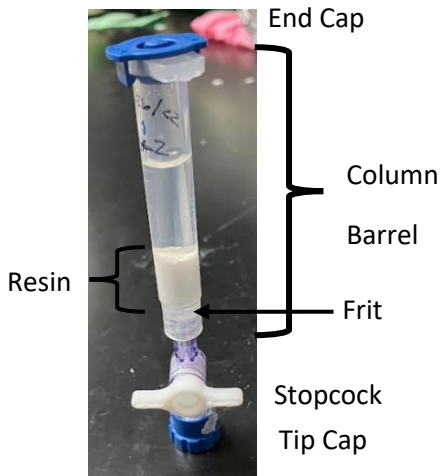


Tip Cap

End Cap

Frits

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Assembled Column

See the 'Column Assembly Protocol' document for instructions on how to assemble the columns.

- Coomassie Protein Assay Reagents
 - Thermo Scientific, #1856209, comes with Coomassie reagent and the 2mg/mL albumin protein standard
- 4mL Falcon tubes – BD Falcon 5mL polypropylene round bottom tubes, 12 x 75mm style, Ref 352063
- Spectrophotometer cuvettes – see Module 2
- Micropipette tips – see Module 2
- 0.5mL Microfuge tubes – available from many scientific vendors