

Downstream Process - Column Purification Protocol

Materials:

1. **Bacterial Lysate:** The tube containing the bacterial solution + lysis buffer that you prepared previously.
2. **p1000 Micropipette tips:** For measuring small volumes of reagents or bacterial cultures
3. **Microfuge tubes of various sizes:** For containing small volumes of reagents or bacterial cultures
4. **Microfuge tube rack:** To hold microfuge tubes.
5. **Liquid waste container:** This is the container will catch all liquids that pass through the column prior to elution of the RFP or GFP.
6. **Sharpie markers:** For correct labeling of samples.
7. **Binding Buffer (BB):** Provides the salt that enhances binding of RFP or GFP to the column resin
8. **Wash Buffer (WB):** A lower salt solution that washes off anything that is not specifically bound to the column.
9. **Elution Buffer (TE):** This is a buffered solution you will resuspend your bacteria in prior to lysis.
10. **Column Equilibration Buffer (CEB):** This solution prepares the column to be used again.
11. **Downstream Process Batch Record Form:** When properly filled out, this form is a record of your entire upstream process.

Equipment:

1. **Chromatography column + ring stand holder**
2. **p1000 Micropipette**
3. **Small tabletop Microcentrifuge**

Protocol:

Column purification will be carried out by the Downstream Process Technician. The Process Engineer will carry out centrifugation steps.

The Downstream Process Technician and Process Engineer should work with their team members to carry out this procedure.

Dispose of all waste properly. **Do not allow the column to run dry.**

You are STRONGLY ENCOURAGED to check off each step below as you complete it.

Column Preparation

1. Set the liquid waste container under the stopcock. Remove the stopper on the column tip. Remove the lid on the top of the column. See Fig. 1.
2. Carefully open the column by turning the stopcock valve and allow the liquid to begin draining into the waste collection container.
3. Close the valve once there is about 1-2 mm of liquid left above the resin bed.

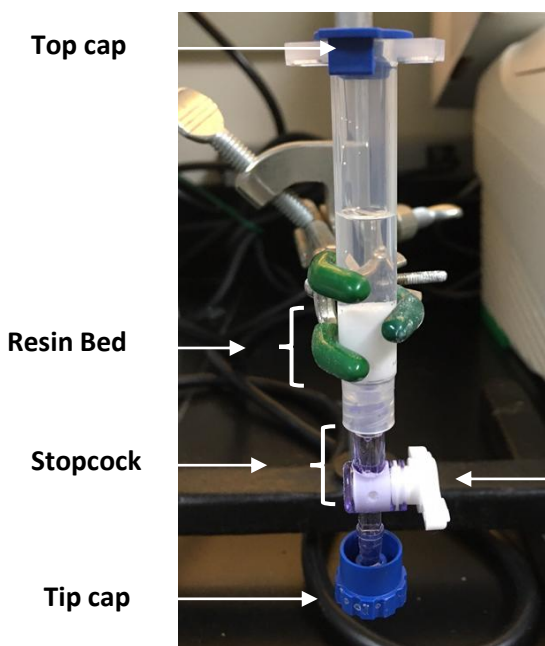
4. Make sure that no liquid is dripping from the column into the waste container.

Column Purification

5. Spin your lysate tube in the microcentrifuge at 6,000 rpm in a small tabletop microcentrifuge for 5 minutes to pellet cell debris.
NOTE: Distribute tubes evenly in the microcentrifuge so that their weight is balanced.
6. After spinning, examine the tube. You should see supernatant (liquid) and a solid pellet.
What color is the supernatant? What color is the pellet? What is contained in the supernatant?
7. Using a p1000 micropipette, carefully remove 200uL of the lysate supernatant without disturbing the cell debris pellet. Dispense the supernatant into a tube labeled Lysate Super.
8. Keep the rest of the spun lysate and store it in the refrigerator.
9. Using a new tip, add 200uL of BB to the Lysate Super tube. Mix gently by pipetting the solution up and down.
10. Add all 400uL of this mixture to the chromatography column. Carefully dispense the solution down the side of the column to avoid disturbing the surface of the resin bed. See Fig. 1 at the end of the protocol to view a photo of a column with all parts labeled.
11. Open the valve and allow the solution in the column to drain into the waste collection container. Close the valve once there is about 1-2 mm of liquid left above the resin bed.
12. Examine the column and locate the red (or green) fluorescent protein. Is it spread throughout the resin bed? Or does it appear to be restricted to a single band?
13. Using a new tip, add 1,000 uL (1 mL) of WB gently down the side of the column.
14. Open the valve and allow the solution in the column to drain into the waste collection container. Close the valve once there is about 1-2 mm of liquid left above the resin.
15. Examine the column and locate the red fluorescent protein. Has the location of the RFP changed in the resin bed?
16. Arrange three 1.5mL microfuge tubes in a microfuge rack. Position one of the tubes under the column stopcock.
17. Using a new tip, add 1,000 uL of TE (Elution Buffer) twice (2 mL total), gently down the side of the chromatography column.

18. With a microfuge tube under the stopcock, open the valve and allow the TE to begin to pass through the column. Does the RFP (or GFP) begin to move?
19. Once you see the RFP get near the bottom of the column, close the valve. Label the next microfuge tube P-RFP, for purified RFP, (or P-GFP for purified GFP) and set it under the stopcock.
20. Open the valve and allow the RFP (or GFP) to drip into this tube. Once the red (or green) has disappeared from the column, close the valve. Measure the volume of your purified RFP (or GFP) and record it on the Downstream Process Batch Record.
21. Cap the P-RFP tube and add your initials to it. It will be stored in the refrigerator until you are ready to determine the protein concentration.
22. Set the waste container back under the stopcock. Open the valve and allow the last of the TE to drip through the column. Close the valve once there is 1-2 mm of liquid above the resin bed.
23. Using a new tip, add 1,000 μ L of CEB twice (2 mL total) to the chromatography column to prepare it for reuse.
24. Allow the CEB to drip through the column. Close the valve when there is about 10mm of liquid above the resin bed. Replace the caps on both ends of the column.
25. Pour the contents of the waste collection container down the sink.
26. Store your RFP (or GFP) tube in the refrigerator. Save the rest of your spun bacterial lysate in the refrigerator as well.
27. Fill out the appropriate parts of the Downstream Process Batch Record.

Column with both ends capped.



Column with both ends un-capped.

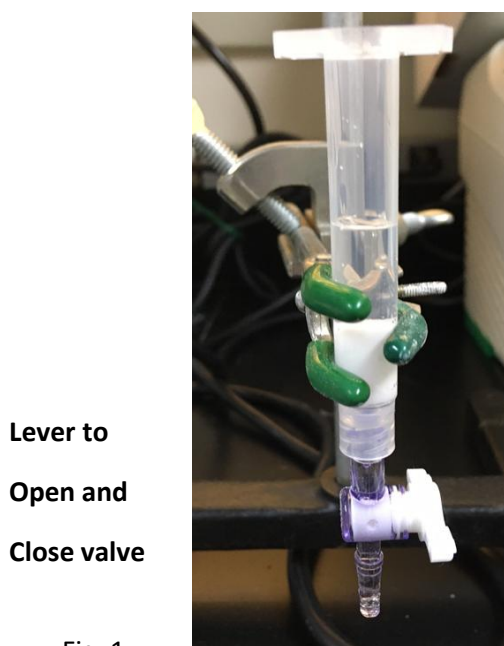


Fig. 1