Buffer Exchange

Project: iGEM2026
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THURSDAY, 9/24/2026

1. **Prepare equilibration Buffer**

Sodium Phosphate buffer (Imidazole free):

1. Weigh out 0.58g sodium phosphate monobasic.
2. Weigh out 1.08g sodium phosphate dibasic anhydrous.
3. Add 230ml Milliq
4. Perform PHing: Add acid/base to achieve PH 7.4.
5. Filll to 250ml total volume with Milliq

2**. Run desalting column**

Zeba™ XL Spin Desalting Columns, 40K MWCO

1. Remove the column's bottom closure. Loosen the cap (do not remove cap).
2. Place the column into a collection tube then centrifuge to remove half of the storage solution.
3. Discard flowthrough then replace the column back into the collection tube and centrifuge again to remove the rest of the storage solution.
4. Discard flowthrough then replace the column back into the collection tube.
5. Add 10 mL of wash/equilibration buffer on top of the resin. Centrifuge tube then discard flowthrough. Repeat for a total of 3 washes and spins. IMPORTANT! Incomplete centrifugation can result in poor sample recovery or sample dilution.
6. Transfer the column to a new collection tube.
7. Apply sample on top of the resin.
8. Centrifuge then retain flowthrough that contains sample. Discard the spin column.

image.png

Table 1 Centrifugation times and volumes for the buffer and sample.

		Column
Sample volume range		4–10 mL
Wash/equilibration buffer volume		10 mL
Centrifuge speed		700 x *g*
Centrifugation time	Storage solution removal #1	2 minutes
	Storage solution removal #2	2 minutes
	Wash 1	3 minutes
	Wash 2	3 minutes
	Wash 3	5 minutes
	Sample recovery	4 minutes

https://documents.thermofisher.com/TFS-Assets/LSG/manuals/MAN1000342-ZebaXLSpinDesaltingColumns-40K-MWCO-UG.pdf

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Introduction

Analytical technique to measure protein sizes.

Materials

- 2X Laemmli Buffer
- 1X Binding Buffer
- Heat Block
- SDS-PAGE Apparatus
- Gel Cassette
- 1X Running Buffer
- Coomassie Dye
- Power Supply
- Large Weighing Boat

Procedure

Resuspension of Insoluble Fractions

1. Use an empty falcon tube as a blank to tare scales.
2. Weigh all pellets on the scales.
3. Resuspend the pellets in 1X Binding Buffer at 10mL/g. Mix gently. Be careful to not suck up pelleted solids.
4. If the resuspension is very concentrated, consider making a 1:1 dilution - 0.5mL resupension : 0.5mL 1X Binding Buffer.

Sample Preparation

5. Mix 0.5mL sample (supernatant or resuspension) with 0.5mL 2X Laemmli Buffer.
6. Heat for 10 minutes at 95degC using a heat block.

Setup

7. Setup the apparatus with the gel cassette.
8. Pour 1X Running Buffer into the central chamber to the top. Ensure no leakage into other chambers to determine that the setup has been put together properly.
9. Add some 1X Running Buffer into the outer chambers to complete the electrical circuit.
10. Carefully remove the top of the gel cassette to reveal the wells.

SDS-PAGE

11. Pipette 5uL of protein ladder into the leftmost well.
12. Pipette 10uL of each sample into the remaining wells.
13. Attach the lid to the apparatus, making sure the metal prongs are aligned correctly.
14. Connect the cable from the lid to the power supply, ensuring the cables are in the right orientation.
15. Set the power supply to constant voltage, 200V, 40 minutes, and click start. Make sure you can see some dust releasing from the bottom of the gel, indicating that current is flowing.
16. Check up on the gel every so often to ensure that the protein does not slide off of the gel. If the protein is not low enough, consider activating the power supply for longer.
17. Once the protein position is ideal, turn off the power supply and remove the lid.

Analysis

18. Carefully remove the gel cassette.
19. Using a spatula, crack open the plastic cassette to expose the gel.
20. Careful remove the gel from the plastic and place into a large weighing boat. This may require cutting the perimeter of the gel with the spatula.
21. Rinse off the gel with MilliQ water.
22. Cover the gel with a shallow pool of Coomassie Dye.
23. Place the boat on the shaker and turn it on.

- [x] 24. After 25 minutes (or when the bands are clearly visible), pour the Coomassie Dye into a Falcon tube and replace it with MilliQ water. It can now be kept and later imaged.