nanoComposix

Antibody Purification Protocol

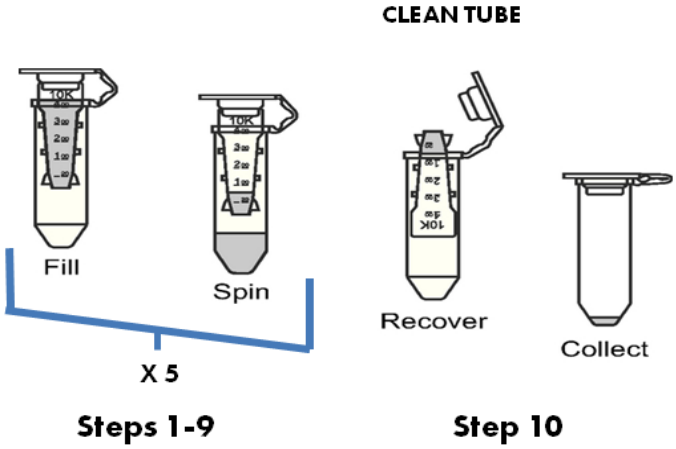
1. INTRODUCTION

It is critical to purify proteins (e.g. antibodies) before conjugation to nanoparticles. Commercial antibodies may contain salts and protein additives for stabilization (e.g. BSA), amines as a preservative (e.g. sodium azide), or amines in the buffer (e.g. Tris) that need to be removed before conjugation. The filters provided have a 10 kDa molecular weight cut-off for antibody purification and are suitable for purifying or concentrating proteins larger than 10 kDa, while filtering out free amines such as sodium azide or transferring to a suitable buffer. These filters will not remove unwanted stabilizing proteins >10 kDa, such as BSA. Use appropriate columns to remove stabilizing proteins if needed. If Tris buffer is required to remove stabilizing proteins, the antibody will need to be purified a second time to transfer it to a suitable amine-free buffer.

2. MATERIALS

- Millipore Amicon Ultra 0.5mL Filters for Protein Purification and Concentration 10K (Catalog# UFC501096)
- 2 mL Microcentrifuge tubes (to hold filters)
- Microcentrifuge
- Antibody to be purified
- Antibody purification buffer
    - 10 mM potassium phosphate, pH 7.4
- BCA assay kit and plate reader or UV-vis spectrophotometer for protein quantification

3. ANTIBODY PURIFICATION



1. Place filter inside microcentrifuge tube.
2. Pre-wet filter by adding 450 µL of your desired buffer. Centrifuge 5 minutes at 13.8k RCF.

   NOTE: *An extra filter setup or eppendorf tube of similar mass will be required as a counter balance in the centrifuge.*

3. Dispose of filtrate.
4. Aliquot antibody solution into filter and close cap.

   NOTE: *Most antibodies are stable at room temperature for approximately 45-60 minutes. Be sure to check the Certificate of Analysis for your particular antibody. If the antibody is not stable, a refrigerated centrifuge may be required.*

   NOTE: *The filter can hold up to 500 µL. If the volume to purify exceeds this capacity, you can spin to concentrate and add more unpurified antibody before continuing with the wash steps. If the starting antibody volume is minimal, make up the volume by adding buffer up to ~450 µL total volume.*

5. Centrifuge 5 minutes at 13.8k RCF to concentrate.
6. Remove the assembled device from the centrifuge and remove the filter from the microcentrifuge tube. Remove filtrate from bottom of tube.
7. Place the filter containing the concentrated antibody back into the tube and add 350 µL purification buffer (e.g. 10mM potassium phosphate or other amine-free buffer).
8. Centrifuge for 5 minutes at 13.8k RCF to wash/concentrate.
9. Repeat washing procedure (steps 5-7 above) an additional four times using 350 µL of additional purification buffer for a total of five washes.
10. After the final wash, turn device upside down in a ***new, clean*** 2 mL microcentrifuge tube (provided). Centrifuge 5 minutes at 1k RCF to collect purified and concentrated antibody

    NOTE: *For optimal recovery, perform the reverse spin immediately. The cap may be cut off with scissors before spinning.*

11. Bring to final volume required so that your antibody concentration is ≥1 mg/mL for storage.

    NOTE: *After purification, we typically recommend aliquoting and storing purified antibodies ≥1 mg/mL at -20° C and avoiding freeze/thaw cycles. However, antibody stability varies. Always refer to antibody supplier for proper storage and handling.*

4. DETERMINING FINAL PROTEIN CONCENTRATION

Use A_{280} method or BCA assay to confirm concentration of starting material and final purified material to determine yield.

5. MAXIMIZING SAMPLE RECOVERY

Low sample recovery in concentrate may be due to adsorptive losses, over-concentration, or passage of sample through the membrane. To maximize sample recovery:

- Ensure that the pipette tip does not puncture the membrane filter.

  NOTE: *you may choose to retain filtrate from all wash steps in a clean container. If yield is particularly low due to punctured filter, you may reclaim antibody from retained filtrate by repeating Section 3 with a new filter.*

- Adsorptive losses depend upon solute concentration, hydrophobic nature, temperature, time of contact with filter device surfaces, sample composition, and pH. To minimize losses, remove concentrated samples immediately after centrifuging.
- If the starting sample concentration is high, monitor the centrifugation process in order to avoid over-concentration of the sample. Over-concentration can lead to precipitation and potential sample loss.
- If the sample appears to be passing through the membrane, choose a lower nominal molecular weight limit (NMWL) Amicon Ultra-0.5 filter unit.
- After collecting concentrated/purified antibody, you may choose to rinse the inside of the filter a few times with a small volume of purification buffer to reclaim any of the antibody that may be on the filter membrane.

6. ADDITIONAL RESOURCES

For technical support, contact (858) 565-4227 x2 or email us at info@nanocomposix.com.