

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 by nanoComposix (Millipore-Sigma Cat # UFC5010) 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 small molecules and 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 affinity columns to isolate the antibody from stabilizing proteins if needed. If Tris or another amine containing buffer is used to elute the antibody from the affinity column during isolation from stabilizing proteins, the antibody will need to be purified a second time to transfer it to a suitable amine-free buffer.

It is important to note that the antibody purification referred to in this procedure is different than affinity purification during antibody development and processing. Although the antibody may have been affinity purified, it needs to be purified and transferred into a suitable buffer free of additional amines. Even a small concentration of sodium azide (NaN_3) will interfere with the efficacy of conjugation.

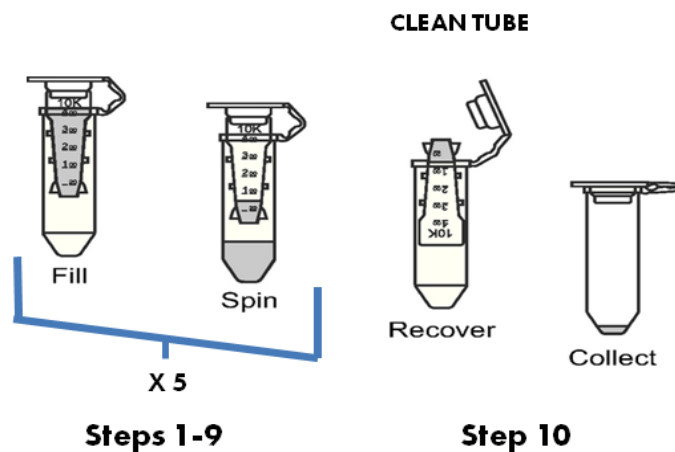
2. KIT COMPONENTS

1. Antibody Purification Filters
 - Millipore Amicon[®] Ultra 0.5mL Filters 10kDa (Catalog# UFC501096)
2. 2 mL Microcentrifuge tubes
3. Antibody purification buffer
 - 10 mM potassium phosphate, pH 7.4

3. ADDITIONAL MATERIALS NEEDED

- Microcentrifuge
- Antibody to be purified
- BCA assay kit and plate reader or UV-Vis spectrophotometer for protein quantification

4. ANTIBODY PURIFICATION



1. Place the filter inside of the microcentrifuge tube.
2. Pre-rinse filter by adding 450 μL of your desired buffer. Centrifuge 5 minutes at 13.8k RCF.
3. Dispose of filtrate.
4. Aliquot antibody solution into filter and close cap.

NOTE: The filter can hold up to 500 μL . If the volume to purify exceeds this capacity, you can centrifuge to concentrate and add additional 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.

NOTE: *There will be a small amount of solution that does not go through the membrane. This contains the antibody. Spinning until all the solution has gone through may cause adsorptive loss of antibody on the membrane.*

6. Remove the assembled device from the centrifuge and remove the filter from the microcentrifuge tube. Discard filtrate from bottom of tube.
7. Place the filter containing the concentrated antibody back into the tube and add 350 μ L purification buffer to the filter (e.g. 10mM potassium phosphate or another 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 μ L 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).
11. 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.*

12. Bring to final volume required so that your antibody concentration is ≥ 1 mg/mL for storage (recommended).
13. After purification, it may be useful to aliquot and store purified antibodies ≥ 1 mg/mL at recommended storage temperature and to minimize freeze/thaw cycles. Antibody stability and storage conditions vary. Always refer to the data sheet provided by the antibody supplier for proper storage and handling.

5. DETERMINING FINAL PROTEIN CONCENTRATION

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

6. 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:

- Most antibodies are stable at room temperature for approximately 45-60 minutes. Be sure to check the data sheet for your particular antibody, or speak with the supplier. If the antibody is not stable, a refrigerated centrifuge may be required.
- Ensure that the pipette tip does not puncture the membrane filter. 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 centrifugation.
- If the starting sample concentration is high, monitor the centrifugation process 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.

7. ADDITIONAL RESOURCES

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