Protocol

Ammonium Sulfate Fractionation of Antibodies

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Before the widespread availability and use of Protein A (rabbit) or Protein G (rodent) for the purification of IgG, the use of an ammonium sulfate "cut" was the standard method to isolate IgG and other serum proteins. The addition of ammonium sulfate reduces the effective solubility of proteins through direct competition for binding sites on the surface of the protein. The resulting precipitated proteins can be isolated by centrifugation. An ammonium sulfate concentration between 40% and 50% results in the precipitation of IgG from most species, and thus 50% is usually used. Because other proteins can be "trapped" within the aggregated protein, the use of ammonium sulfate does not result in a purified antibody fraction and as such should be considered a first step in a multistep antibody purification protocol.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Ammonium sulfate, saturated <R> (>99% purity)
Phosphate-buffered saline (PBS) <R>
Serum, ascites, or cell culture medium (supernatant)
Tris buffer (10 mM, pH 6.5) <R>

Equipment

Centrifuge, refrigerated (or located in a cold room), of sufficient capacity to centrifuge the volume being processed
Dialysis tubing (10- or 25-kDa MWCO)
Filter (0.2 µm)

Use either a bottle type, Nalgene, or syringe filter. The choice will depend on the volume to be processed.

pH meter
Stir plate
UV spectrophotometer

From the Antibodies collection, edited by Edward A. Greenfield.

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METHOD

1. Remove insoluble material from the serum, ascites, or cell culture supernatant by centrifugation at 3000*g* for 30 min at 4°C.
2. Transfer the supernatant to a beaker, and *slowly* add saturated ammonium sulfate to a final concentration of 25% (w/v).
3. Incubate for 5–15 h at 4°C.
4. Centrifuge at 3000*g* for 30 min at 4°C.
5. Carefully decant the supernatant into a fresh beaker.
6. Add saturated ammonium sulfate (one-third of the supernatant volume) to bring its final concentration to ~50%.
7. Incubate for 5–15 h at 4°C.
8. Centrifuge at 3000*g* for 30 min at 4°C.
9. Remove the supernatant. Carefully resuspend the pellet in 30%–50% of the original volume. Transfer the entire sample to dialysis tubing (10- to 25-kDa MWCO).

   Avoid excessive frothing, and use dialysis tubing that can hold twice the sample volume to allow for expansion of the solution during dialysis.

10. Dialyze against at least three changes of 1× PBS for 24–48 h. Any flocculent material can be removed by centrifugation. If, however, the antibody will be further purified by ion-exchange chromatography (e.g., DEAE), a lower ionic strength dialysate should be used, such as 10 mM Tris buffer (pH 6.5) or similar buffer.
11. When dialysis is complete, determine the protein concentration by measuring the optical density at 280 nm. For IgG, an A_{280} of 1.35 = 1 mg/mL.

    This crude antibody fraction can be further purified by a variety of methods, including Protein A/G purification, anion-exchange chromatography using diethylaminoethyl (DEAE)–Sepharose, or other methods (affinity chromatography, size exclusion, or other chromatographic methods). If the IgG fraction is to be stored for any length of time without further workup, it should be filtered through a 0.45-µm filter and stored at 4°C.

RECIPES

Ammonium Sulfate, Saturated

1. Add 761 g of ammonium sulfate (for research grade antibodies, use Sigma-Aldrich catalog no. A2939 or equivalent) to 1 L of appropriate grade H_2O (~4.1 M).
2. Dissolve with stirring and gentle heating.
3. Cool to room temperature and adjust the pH to 7 (with NH_4OH or an acid such as HCl).
4. If excess is to be stored, sterilize the solution by filtering through a 0.2-µm filter and store at 4°C.

Phosphate-Buffered Saline (PBS)

Reagent	Amount to add (for 1× solution)	Final concentration (1×)	Amount to add (for 10× stock)	Final concentration (10×)
NaCl	8 g	137 mM	80 g	1.37 M
KCl	0.2 g	2.7 mM	2 g	27 mM
Na_2HPO_4	1.44 g	10 mM	14.4 g	100 mM
KH_2PO_4	0.24 g	1.8 mM	2.4 g	18 mM
If necessary, PBS may be supplemented with the following:				
$CaCl_2 \cdot 2H_2O$	0.133 g	1 mM	1.33 g	10 mM
$MgCl_2 \cdot 6H_2O$	0.10 g	0.5 mM	1.0 g	5 mM

PBS can be made as a 1× solution or as a 10× stock. To prepare 1 L of either 1× or 10× PBS, dissolve the reagents listed above in 800 mL of H_2O. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H_2O to 1 L. Dispense the solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle or by filter sterilization. Store PBS at room temperature.

Tris Buffer (10 mM, pH 6.5)

Reagent	Quantity (for 1 L)
NaCl	8 g
KCl	0.2 g
Tris	12.1 g

Dissolve the above materials in 800 mL of distilled H_2O (or equivalent). Adjust the pH to 6.5 with HCl, and bring the volume to 1000 mL.

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