

Purification of IgG by Precipitation with Sodium Sulfate or Ammonium Sulfate

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1. Introduction

Addition of appropriate amounts of salts, such as ammonium or sodium sulfate, causes precipitation of IgG (*I*) from all mammals, and can be used for serum, plasma, ascites fluid, and hybridoma culture supernatant. Although such IgG is usually contaminated with other proteins, the ease of these precipitation procedures coupled with the high yield of IgG has led to their wide use in producing enriched IgG preparations. They are suitable for many immunochemical procedures, e.g., production of immunoaffinity columns, and as a starting point for further purification. It is not suitable however for conjugating with radiolabels, enzymes, or biotin since the contaminating proteins will also be conjugated, thereby reducing the efficiency of the labeling and the quality of the reagent. The precipitated IgG is usually very stable, and such preparations are ideally suited for long-term storage or distribution and exchange between laboratories.

Ammonium sulfate precipitation is the most widely used and adaptable procedure, yielding a 40% pure preparation; sodium sulfate can give a purer preparation for some species, e.g., human and monkey.

2. Materials

2.1. Ammonium Sulfate Precipitation

1. Saturated ammonium sulfate solution: Add excess $(\text{NH}_4)_2\text{SO}_4$ to distilled water (about 950 g to 1 L), and stir overnight at room temperature. Chill at 4°C, and store at this temperature. This solution (in contact with solid salt) is stored at 4°C.
2. PBS: 0.14M NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4 , 8.1 mM Na_2HPO_4 . Store at 4°C.

2.2. Sodium Sulfate Precipitation

This requires solid sodium sulfate.

3. Methods

3.1. Ammonium Sulfate Precipitation

1. Prepare saturated ammonium sulfate at least 24 h before the solution is required for fractionation. Store at 4°C.

2. Centrifuge serum or plasma for 20–30 min at $10,000g_{av}$ at 4°C . Discard the pellet (*see* Note 1).
3. Cool the serum or plasma to 4°C , and stir slowly. Add saturated ammonium sulfate solution dropwise to produce 35–45% final saturation (*see* Note 2). Alternatively, add solid ammonium sulfate to give the desired saturation (2.7 g of ammonium sulfate/10 mL of fluid = 45% saturation). Stir at 4°C for 1–4 h or overnight.
4. Centrifuge at $2000\text{--}4000g_{av}$ for 15–20 min at 4°C (alternatively for small volumes of 1–5 mL, microfuge for 1–2 min). Discard the supernatant, and drain the pellet (carefully invert the tube over a paper tissue).
5. Dissolve the precipitate in 10–20% of the original volume in PBS or other buffer by careful mixing with a spatula or drawing repeatedly into a wide-gage Pasteur pipet. When fully dispersed, add more buffer to give 25–50% of the original volume and dialyze against the required buffer (e.g., PBS) at 4°C overnight with two to three buffer changes. Alternatively, the precipitate can be stored at 4 or -20°C if not required immediately.

3.2. Sodium Sulfate Precipitation (*see* Note 3)

1. Centrifuge the serum or plasma at $10,000g_{av}$ for 20–30 min. Discard the pellet, warm the serum to 25°C , and stir.
2. Add solid Na_2SO_4 to produce an 18% w/v solution (i.e., add 1.8 g/10 mL), and stir at 25°C for 30 min to 1 h.
3. Centrifuge at $2000\text{--}4000g_{av}$ for 30 min at 25°C .
4. Discard the supernatant, and drain the pellet. Redissolve in the appropriate buffer as described for ammonium sulfate precipitation (Section 3.1., step 5).

4. Notes

1. If lipid contamination is excessive in ascites fluids, thereby compromising the salt precipitation, add silicone dioxide powder (15 mg/mL) and centrifuge for 20 min at $2000g_{av}$ (2) before adding the ammonium or sodium salt.
2. The use of 35% ammonium sulfate will produce a pure IgG preparation, but will not precipitate all the IgG present in serum or plasma. Increasing saturation to 45% causes precipitation of nearly all IgG, but this will be contaminated with other proteins, including some albumin. Purification using $(\text{NH}_4)_2\text{SO}_4$ can be improved by repeating the precipitation, but this may cause some denaturation. Precipitation with 45% $(\text{NH}_4)_2\text{SO}_4$ is an ideal starting point for further purification steps, e.g., ion-exchange or affinity chromatography and FPLC purification (*see* Chapters 127 and 128).
3. Sodium sulfate may be used for precipitation of IgG instead of ammonium sulfate. The advantage of the sodium salt is that a purer preparation of IgG can be obtained, but this must be determined experimentally. The disadvantages are that yield may be reduced depending on the IgG characteristics of the starting material, IgG concentration, and composition. Fractionation must be carried out at a precise temperature (usually 25°C), since the solubility of Na_2SO_4 is very temperature dependent. Sodium sulfate is usually employed only for the purification of rabbit or human IgG.

References

1. Heide, K. and Schwick, H. G. (1978) Salt fractionation of immunoglobulins, in *Handbook of Experimental Immunology*, 3rd ed. (Weir, D. M., ed.), chap. 7. Blackwell Scientific, Oxford.
2. Neoh, S. H., Gordon, C., Potter, A., and Zola, H. (1986) The purification of mouse MAb from ascitic fluid. *J. Immunol. Methods* **91**, 231.