

Ammonium Sulphate Precipitation Procedure

The Midi Spin column has a maximum volume capacity of 20 ml in a swing bucket rotor. If you have a large volume of sample (> 200 ml), we recommend using the established procedure of ammonium sulphate precipitation to concentrate your target antibody. This technique is known as 'salting-out' at high concentration of salt (which in this procedure is ammonium sulphate). In this case, ammonium sulphate is dissolved into the solution containing the antibody until the protein precipitates out of solution. The salt reduces the solubility of the target protein.

One of the principal decisions to make is the percentage saturation of ammonium sulphate to use. Although many IgGs (γ -globulins) precipitate at a lower concentration of ammonium sulphate than most other proteins, 50 % ammonium sulphate is sufficient. It is difficult to be more precise as many factors such as the properties of other proteins present in the sample can influence the ammonium sulphate concentration required to precipitate your target protein.

Ammonium Sulphate Precipitation:

- (1) Dissolve in 315 g ammonium sulphate per litre of serum or sample, while stirring. This gives 50 % saturation. Ensure that all lumps of ammonium sulphate are broken down and that the last spoonfuls of salt are added slowly to the sample. The amount of ammonium sulphate to add to obtain a pre-determined concentration can be calculated using the following formula. The formula allows for the increase in volume caused by the addition of salt:

Amount of ammonium sulphate (in grams) to add to 1 litre of a solution at 20 °C
$$= 533(S_2 - S_1) / 100 - 0.3S_2$$

S_2 = Final % saturation (E.g. 50 %)

S_1 = Initial % saturation (E.g. 0 %; the starting material being equivalent to water)

- (2) After all the ammonium sulphate has dissolved, stir for another 2 hours to allow complete equilibration between the dissolved and aggregated proteins.
- (3) Centrifuge the sample at 10,000g for 15 mins to pellet the precipitated protein containing your target antibody.
- (4) Decant the supernatant and dissolve the precipitate in a small volume of suitable buffer eg PBS. A volume of buffer twice that of the volume of the precipitate is sufficient.
- (5) The dissolved precipitate contains a lot of ammonium sulphate. This must be removed. Either dialyse against a suitable buffer eg PBS or use gel-filtration/de-salting columns.