Ammonium Sulfate Saturated Solution

Rev. 05/19

(Catalog # 7096-1L; 1L; Sterile; 4.32 M; Store at ambient temperature)

I. Introduction:

Protein solubility is dependent on the ionic strength of the solution, among other factors. Two distinct phenomenon of protein solubilization are seen in general: 1) Salting in: At low salt concentrations, the solubility of the protein increases with increasing salt concentration (i.e. increasing ionic strength). 2) Salting out: As the salt concentration (ionic strength) increases, the solubility of the protein begins to decrease. At certain ionic strength, the protein will be almost completely precipitate from the solution. Since proteins differ markedly in their solubility at high ionic strength, salting-out is a very useful procedure to assist in the purification of a given protein.

Ammonium sulfate has been widely used in salting out for protein purification, as it is very water soluble, forms two ions high in the Hofmeister series[1], and has no adverse effects upon enzyme activity. It is generally used as a saturated aqueous solution which is diluted to the required concentration and expressed as a percentage concentration of the saturated solution (a 100% solution). While ammonium sulfate fractionation is a low resolution technique, it is used successfully to reduce the complexity and decrease the sample volume on a daily basis by many scientists.

II. Application:

- Purification/Fractionation of proteins
- Concentration of protein solutions
- Buffer exchange
- Remove interference of small molecules from protein/enzyme solutions.

III. Sample Type:

- Serum, plasma & other body fluids
- Tissues extracts, animal, plant, bacterial extracts, etc.
- Cell culture supernatants
- Fermentation media

IV. User Supplied Reagents and Equipment:

- Stirrer
- High-speed centrifuge (≥ 5000 x g)

V. Storage and Handling:

Store product at ambient temperature, protected from light.

VI. Reagent Preparation and Storage Conditions:

- Ready to use as supplied. Store at ambient temperature, protected from light.

VII. Protein Precipitation Protocol:

1. **Sample Preparation:** The protein solution should be equilibrated to the temperature at which the precipitation procedure is carried out. There should be no precipitate or debris floating in the solution. Presence of precipitate before addition of ammonium sulfate will not result in reproducible precipitation. If required, centrifuge at ≥ 10,000g at 4°C for 10 minutes.

2. **Procedure:**

   i. Add the desired amount of saturated ammonium sulfate solution with slow stirring. Allow precipitation for the appropriate amount of time (from 30 minutes to overnight). We recommend that you carry out overnight precipitation at 4°C. Salting out is temperature dependent. Precipitation at 4°C generally needs longer, relative to precipitation at ambient temperature.

   ii. Collect the precipitated protein by centrifugation for 30 minutes at ≥ 5,000 g at 4°C. Decant the supernatant. Dissolve pellet (generally in 10 times lower volume than the initial volume) in appropriate buffer. In some cases a higher purity can be achieved by re-suspending the pellet in the ammonium sulfate concentration from the previous step and repetition of the centrifugation step. If the target protein is sensitive to the ammonium sulfate pH and the sample does not have sufficient buffering capacity, titrate the pH of the ammonium sulfate appropriately with 10 N NaOH or 6 N HCl before adding to the sample.

   iii. For optimal conditions screening (when working with new target protein), run parallel precipitation experiments at increasing ammonium sulfate concentration (i.e. 10%, 20%, 30%, etc.). After incubation and centrifugation at ≥ 5,000 g at 4°C for 30 minutes, collect the pellets and analyze for the amount of target protein in all pellets. The final supernatant has very high salt concentration, which can affect biological activity of the target protein (if still present in it). Perform appropriate desalting before analyzing for its biological activity.

   iv. If there is precipitate in the bottle of saturated ammonium sulfate, warm the solution to 22°C. If the precipitate persists, let the salt settle and use the clear supernatant, as it contains saturated concentration of ammonium sulfate in your environment.

VIII. Related Products:

Protein A, G and LAG resins
Heparin Sepharose
EZBlock Inhibitor Cocktails
EZLys(TM) Protein Extraction Reagents
Jacalin Sepharose
Protein Quantitation Kit
BCA Protein Quantitation Kit
EZLys(TM) Lysozyme, Human

IX. References:

1. Hofmeister, F., Arch. Exptl. Pathol. Pharmakol. (1888) **24**, 247
2. Englard, S., Seifter, S., Meth. Enzymol. (1990) **182**, 285-300
3. Chien, A.S., Guzetta, A.W., 52nd ASMS Conference on Mass Spectrometry (2004) ThPW-469

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