Technical Note:
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Large Volume Precipitation of Proteins with Ammonium Sulfate using Thermo Scientific Fiberlite Carbon Fiber Rotors

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KEY WORDS

- **Ammonium Sulfate Precipitation**
- **Carbon Fiber Rotors**
- **Superspeed Centrifuges**

Introduction

Ammonium sulfate precipitation is a method of protein purification by altering the solubility of protein. Ammonium sulfate is commonly used due to its high solubility that allows salt solutions with high ionic strength.

At sufficiently high ionic strength, the protein will be almost completely precipitated from the solution. Differential precipitation of proteins by ammonium sulfate is one of the most widely used preliminary purification procedures. It is based on proteins having differing solubility in ammonium sulfate solutions and can result in a two- to fivefold increase in specific activity.

Provided that appropriately buffered ammonium sulfate solutions are used to protect the desired activity, recoveries approaching 100% can be expected. A typical protocol consists of adding ammonium sulfate to give specific percentage saturation, followed by a period of time for proteins to precipitate and a centrifugation step to collect the precipitate. The protocol shown was carefully followed to precipitate the protein removed by centrifugation using the large volume Thermo Scientific Fiberlite F12-6x500 LEX carbon fiber rotor in lieu of using the smaller rotors reported by Elder et al, 1983.[1] The total capacity sample volume of the Fiberlite® F12-6x500 LEX rotor is 3 liters and is rated for RCF up to 25,000 xg.

The ammonium sulfate concentration was increased to a value that will precipitate most of the protein of interest, while leaving the maximum amount of protein contaminants still in solution. The precipitated protein of interest was recovered by centrifugation and dissolved in fresh buffer for the next stage of purification.

Figure 1: Thermo Scientific Fiberlite F12-6x500 LEX carbon fiber rotor.

Protocol: Ammonium Sulfate Precipitation

Materials

1. Ammonium sulfate
2. 1M HEPES, pH 7.4, OR 1 M Tris-HCl, pH 7.5
3. 1 X PBS , pH 7.2-7.4
4. Tubes to collect clarified protein supernatant, serum, or ascites
5. Equipment and reagents for centrifugation

Method

1. Measure the volume of protein solution to obtain 1500 mL. If necessary add 1 M HEPES, pH 7.4, or 1 M Tris-HCl, pH 7.5, to a final concentration of 50 mM. While gently stirring, slowly add ammonium sulfate to 45% saturation (277 g ammonium sulfate/liter solution). Stir gently for a minumum of 4 hours and a maximum of 16 hours at 4 °C.

Note: Addition of ammonium sulfate acidifies the solution, therefore protein stability may be improved by buffering with at least 50 mM HEPES or Tris buffer. It is important that no foaming of the solution occurs during ammonium sulfate addition as this will cause denaturation of proteins. If foaming occurs, slow the addition of ammonium sulfate. Make sure that the ammonium sulfate dissolves nearly as quickly as it is added.

2. Transfer the ammonium sulfate precipitated solution to 500 mL polycarbonate centrifuge bottles with screw caps. Centrifuge at 25,000 xg for 20 minutes at 4 °C with the Fiberlite F12-6x500 LEX rotor to pellet precipitated proteins.

3. Decant the supernatant, being sure not to disturb the relatively loose protein-ammonium sulfate pellet. At this point, the pellet can be stored for several weeks at 4 °C without significant loss of protein stability. The supernatant can also be stored at 4 °C for later analysis.
4. The pellet can be re-suspended in PBS, pH 7.2. The solution will remain turbid, but discreet flocculent is not visible.
5. To remove residual precipitate, use adapters with 250 mL conical tubes to collect the clarified proteins by centrifugation at 25,000 xg for 10 minutes at 4 °C. Remove the clarified proteins to fresh tubes and store at 4 °C for up to one week.
6. Alternatively, if column purification is going to be performed immediately, dialyze protein against column binding buffer. Pass the solution through a 0.45 µm filter and apply to the appropriate column.

Conclusion

This protocol is useful to quickly remove large amounts of contaminant proteins, as done in the first step in many purification schemes. It is also often employed during the later stages of purification to concentrate protein from a dilute solution following procedures such as gel filtration.[2]

In addition to the rapid precipitation of protein contaminants, the Fiberlite F12-6x500 LEX rotor will harvest bacteria and yeast from cultures in shorter run times than rotors with similar total volumes. Finally, continued use of the alkaline salt solutions (ammonium sulphates and ammonium phosphates) result in corrosion of aluminum alloys. However, carbon fiber rotors offer negligible resistance to these alkaline salt solutions for the lifetime of the rotors.

References

1. G. H. Elder, J. A. Tovey, and D. A Sheppard, Biochem. J., 215, 45-55 (1983).
2. Ute Koch, Swati Choksi, Lisa Marcucci and Robert Korngold Journal of Immunology, 1998, 161: 421-429.

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