

## Ammonium Sulfate PPT Protocol



**Theory and Introduction: Ammonium Sulfate Precipitation** is a classic first step to fractionate proteins by causing perturbations in the solvent with respect to ionic strength. Historically, separation methods were limited and as a result precipitation methods were highly used with very fine cuts in ppt conditions. As more choices of inexpensive and quality resins are commercially available precipitation steps are typically limited to one or two initial cuts in the beginning of purification or simply used to concentrate the proteins. The major advantage to  $(\text{NH}_4)_2\text{SO}_4$  precipitation is that it easily causes the reversible precipitation of the protein and is non-denaturing to the protein structure.

### **Important Points to Consider For Ammonium Sulfate Precipitation.**

- Addition of solid - Add the solid slowly. Simply dumping in the salt at one time will cause the initial concentration to be much higher as the solid dissolves, resulting in the wrong protein to be precipitated. Add the solid  $\frac{1}{4}$  at a time while stirring on a stir plate. Conducting this in the cold room. Avoid frothing of your solution, this indicates denatured protein at the water-air interface.
- Tables of Ammonium Sulfate Addition - There are tables available (see the lab webpage for links) to tables to use for fine-tuning your ammonium sulfate precipitations.

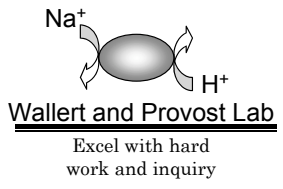
### **General Protocol for MGH Fractionation Using Ammonium Sulfate Precipitation -**

1. First you will perform a 30 % cut of the ammonium sulfate. Some proteins will precipitate at this point, MGH may or may not. You will not know until this is performed experimentally.
  - a. Slowly add solid ammonium sulfate to a final concentration of 30% (179 g / liter of solution) and stir at room temp for 15 min.
  - b. Centrifuge in the 50 ml tubes at max speed in the biochem lab for 15 min.
  - c. Separate the supernatant solution from the pellet. Resuspend the pellet in 1 ml of a simple buffer.
2. Measure the supernate from step one and perform a 70% ammonium sulfate precipitation by adding 273 g of solid ammonium sulfate per liter of supernatant solution. Stir and centrifuge as above. Save the pellet and resuspend in 1-2 ml of buffer.
3. Measure for MGH in the each pellet and supernatant solution to determine where the protein is located.

### **General Protocol for MGH Concentration Using Ammonium Sulfate Precipitation -**

4. First you will perform a 80 % cut of the ammonium sulfate. Most proteins will precipitate at this point.
  - a. Slowly add solid ammonium sulfate to a final concentration of 80% (525 g / liter of solution) and stir at room temp for 15 min.
  - b. Centrifuge in the 50 ml tubes at max speed in the biochem lab for 15 min.
  - c. Separate the supernatant solution from the pellet. Resuspend the pellet in an appropriate volume of your buffer of choice.
  - d. MGH will be located in the pellet

**Alternate Method of purification** - An different concentration method involves centrifugation through special filters to retain protein of a set molecular weight but allow the buffer to pass through. This is called ultrafiltration. This is a possible but expensive option. See your instructor for further information.



## **Ammonium Sulfate PPT** Protocol

