

## Precipitation of immunoglobulin (Lab6)

### **Protocol**

1- Allow serum or ascetic fluid to thaw, and centrifuge at 3000g for 30 minutes.

2- Transfer sample to beaker containing stir bar and place on magnetic stirrer.

3- While sample is stirring; slowly add saturated ammonium sulfate to bring final concentration to 50% saturation (**why**)?

- a. Volume of ammonium sulfate is equal to volume of sample
- b. Prevent exceeding the desired concentration.

4- Total volume ammonium sulfate is added, move beaker at 4°C for 6 hours or overnight.

5- Transfer to conical tube and centrifuge the precipitate at 3000g for 30 minutes.

6- Carefully remove and discard supernatant, invert conical tube and drain well.

**Note** : for serum resuspend pellet in 30%-50% of the starting volume in 1x(PBS) while for monoclonal antibody tissue culture supernatants , resuspend pellet in 10% of the starting volume in 1x(PBS).

7- Transfer antibody solution to dialysis tube (**why**)?

To get rid of the ammonium sulfate residues that has been precipitate.

8- Remove antibody solution from tubing and centrifuge to remove any remaining debris.

9- Determine the concentration and store at -80°C for long term storage

**Clinical uses of intravenous immunoglobulin**

- 1- Treatment of neurological disorder including dermatomyositis, Guillain Barre Syndrome, and chronic inflammatory.
- 2- In hematology it is used to treat cytopenia, and chronic lymphatic leukemia
- 3- In immunology is used to treat systemic lupus erythematosus
- 4- In dermatology is used to treat Kawasaki syndrome
- 5-Nephrology is used to treat Vasculitis.