**Antibody Separation**

Antibody separation can be achieved by different means which are listed below:

**1. Precipitation with Ammonium Sulfate**

Protein precipitation achieves separation by the conversion of soluble proteins to an insoluble state, which subsequently can be removed by various means. Ionic precipitation, utilizing inorganic salts like ammonium sulfate, is the most common precipitation method. Ammonium sulfate has several advantages:

• At saturation, it is of sufficiently high molarity that it causes the precipitation of most proteins.

• Its saturated solution has a density that does not interfere with the sedimentation of most precipitated proteins by centrifugation.

• Its concentrated solutions are generally bacteriostatic.

• In solution, it protects most proteins from denaturation.

The concentration of ammonium sulfate required for precipitation varies from protein to protein and should be determined empirically.

**2. Affinity Chromatography using Protein A/ Protein G**

Protein A is a cell wall component produced by several strains of *Staphylococcus aureus*. It has the ability to specifically bind to the Fc region of immunoglobulinmolecules, especially IgG (**Figure 4**). The interaction between Protein A and IgG isnot equivalent for all species, and even within a species, Protein A interacts withsome IgG subgroups and not with others.

Protein G is a bacterial cell wall protein isolated from group G streptococci. Like Protein A from *Staphylococcus aureus*, Protein G binds to most mammalian immunoglobulin through their Fc regions. The unique immunoglobulin binding characteristics of Protein G can be used for the purification of mammalian monoclonal and polyclonal antibodies that do not bind well to Protein A. Protein G is reported to bind with greater affinity to most mammalian immunoglobulin that does Protein A. It also binds with significantly greater affinity to several immunoglobulin subclasses. 

Agarose or Sepharose covalently coupled to Protein A or Protein G can be used as an affinity support for the isolation of immunoglobulin molecules.

Serum is loaded onto a column which is filled with Sepharose coupled to protein so that the immunoglobulin present in the serum can be bound. The rest of the serum proteins will flow through and the immunoglobulin can be eluted with an acidic buffer (**Figure 5**).



**3. Labeling of antibodies**

In the past immunoassays were developed, in which radioactive markers coupled to antibodies or antigens were used for the detection of antibody-antigen interactions. Nowadays, other markers for the labeling of antibodies are used because of safety concerns and because of emerging technologies. Due to their good sensitivity, many antibodies are labeled with enzymes which render a colorless substrate into an easily detectable product. As the amount of colored product is proportional to the enzyme concentration and at the same time to the antigen concentration, it is possible to quantify antibody or antigen concentration and to characterize binding affinities. The basic principle of the method is shown in the figure below (**Figure 6**):

