IMMUNOPRECIPITATION

Immunoprecipitation (IP) is a technique that is routinely used to determine the molecular weight of protein antigens, study protein – protein interactions, determine specific enzyme activity, monitor post-translational modification and quantify proteins. The IP technique allows the detection of rare proteins which would be otherwise difficult to detect, as they can be concentrated up to 10 000-fold by immunoprecipitation.

The technique involves the following steps:

1. To crude cell lysate sample (0.1 – 1 ml) add 10 µl of antibody at appropriate dilution.
2. Incubate for 60 minutes to overnight at 4°C, gently mixing the sample.
3. Add 40 – 100 µl protein A coated beads coated in proteins A or G (for example), approx 25 – 50 µl agarose / bed volume.
4. Incubate for a further 15 – 60 mins at 4°C, gently mixing the sample.
5. Collect immunoprecipitated complexes by centrifugation and discard the supernatant.
6. Wash the pellet with 1 ml of washing buffer by resuspension and centrifugation. Repeat this step at least 3 to 6 times.

Schematic diagram of Immunoprecipitation (Indirect)