# Chaudhary Mahadeo Prasad College

**(A CONSTITUENT PG COLLEGE OF UNIVERSITY OF ALLAHABAD)**

## E-Learning Module

## Subject: Botany

**(Study material for Post Graduate Students)**

## M.Sc. II Sem

### COURSE CODE: BOT 605

### Molecular Biology and Molecular techniques

## Unit (V): Topic: Protein Protein interaction

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## Protein – Protein interaction analysis

Two major methods for analyzing protein-protein interaction are:

1) Co-immunoprecipitation
2) Pull Down assay

## Co-immunoprecipitation (co-IP)

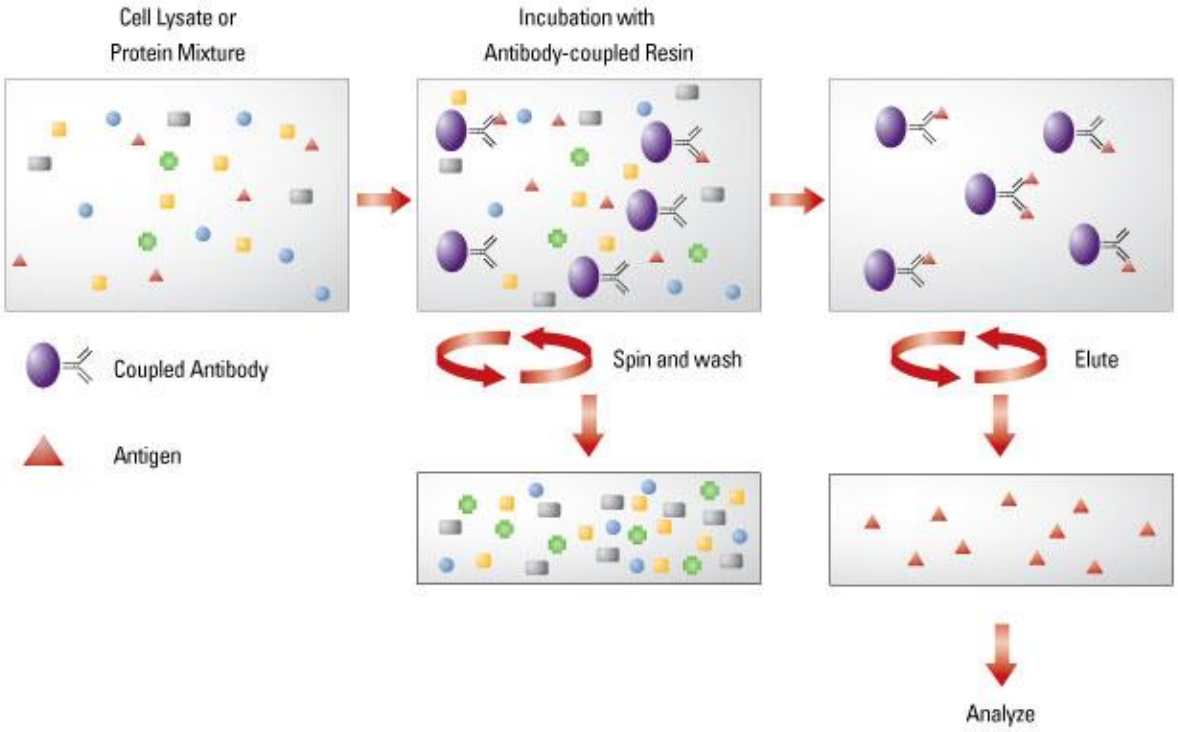
Co-immunoprecipitation (co-IP) is a popular technique to identify physiologically relevant protein–protein interactions by using target protein-specific antibodies to indirectly capture proteins that are bound to a specific target protein. These protein complexes can then be analyzed to identify new binding partners, binding affinities, the kinetics of binding and the function of the target protein.

### Immunoprecipitation (IP) vs. co-immunoprecipitation (co-IP)

The topic of co-immunoprecipitation (co-IP) is best preceded by an overview of immunoprecipitation (IP) to help frame an understanding of the principles involved. The description of IP methodology here is brief.

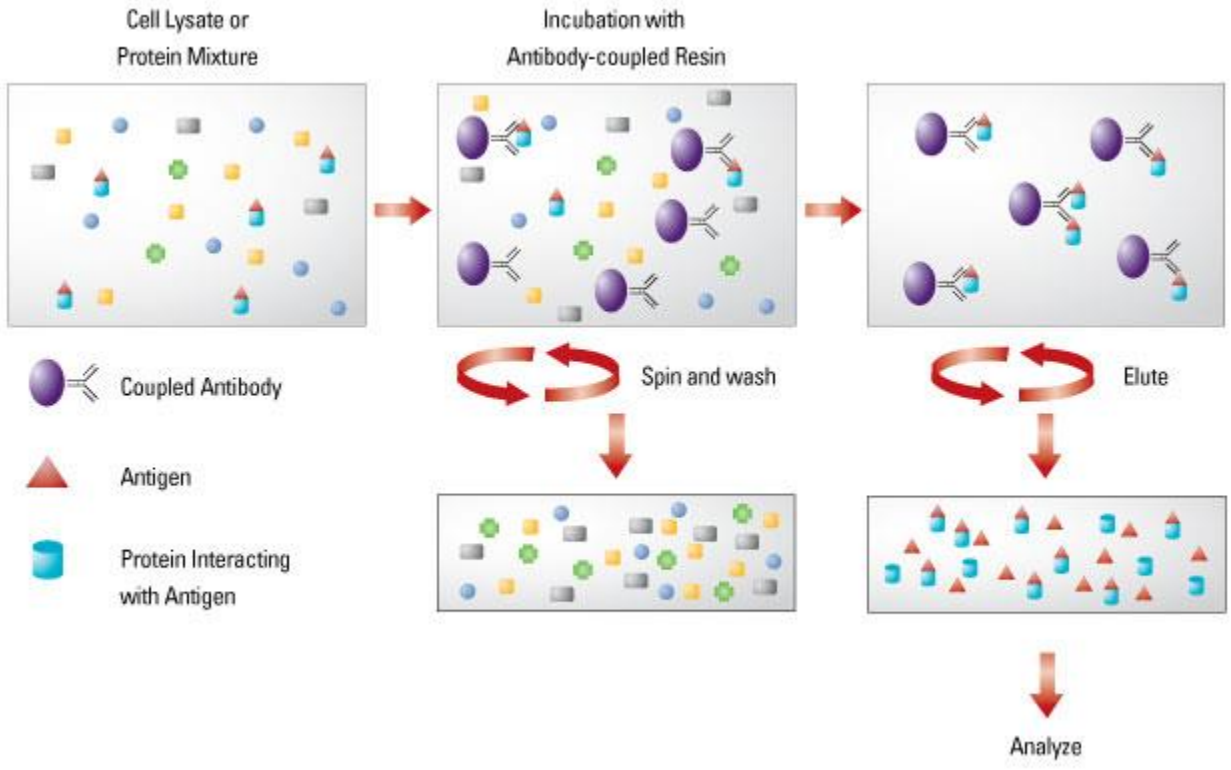
### Immunoprecipitation

Immunoprecipitation is one of the most widely used methods for antigen detection and purification. The principle of an IP is very straightforward: an antibody (monoclonal or polyclonal) against a specific target protein forms an immune complex with that target in a sample, such as a cell lysate. The immune complex is then captured, or precipitated, on a beaded support to which an antibody-binding protein is immobilized (such as Protein A or G), and any proteins not precipitated on the beads are washed away. Finally, the antigen (and antibody, if it is not covalently attached to the beads and/or when using denaturing buffers) is eluted from the support and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), often followed by western blot detection to verify the identity of the antigen.




**Co-immunoprecipitation (co-IP)**

Co-immunoprecipitation is an extension of IP that is based on the potential of IP reactions to capture and purify the primary target (i.e., the antigen) as well as other macromolecules that are bound to the target by native interactions in the sample solution. Therefore, whether or not an experiment is called an IP or co-IP depends on whether the focus of the experiment is the primary target (antigen) or secondary targets (interacting proteins).




## Pull-down assays

The pull-down assay is an *in vitro* method used to determine a physical interaction between two or more proteins. Pull-down assays are useful for both confirming the existence of a protein–protein interaction predicted by other research techniques (e.g., co-immunoprecipitation) and as an initial screening assay for identifying previously unknown protein–protein interactions.

Pull-down assays are a form of affinity purification and are similar to immunoprecipitation, except that a "bait" protein is used instead of an antibody. Affinity chromatography (i.e., affinity purification) methodologies greatly enhance the speed and efficiency of protein purification and simultaneously provide the technology platform to perform a pull-down, or co-purification, of potential binding partners. In a pull-down assay, a bait protein is tagged and captured on an immobilized affinity ligand specific for the tag, thereby generating a "secondary affinity support"' for purifying other proteins that interact with the bait protein. The secondary affinity support of immobilized bait is then incubated with a protein source that contains putative "prey" proteins, such as a cell lysate. The source of prey protein at this step depends on whether the researcher is confirming a previously suspected protein–

protein interaction or identifying an unknown interaction. The method of protein elution depends on the affinity ligand and ranges from using competitive analytes to low pH or reducing buffers.

Besides investigating the interaction of two or more proteins, pull-down assays are a powerful tool to detect the activation status of specific proteins. For example, proteins that are activated in response to tyrosine phosphorylation can be pulled down using an immobilized SH2 domain that targets the phosphorylated tyrosine on a given protein. Additionally, GTPases, which act as molecular switches that regulate cell signaling by cycling between a GTP-bound (active) and GDP-bound (inactive) state, can be pulled down using an immobilized GTPase-binding domain of downstream proteins that are recruited to GTP-bound, activated GTPases. In both types of pull-down assays, because the specificity of the interaction is dependent on the sequence of the binding domain, these approaches are highly specific in detecting the activation of distinct proteins.