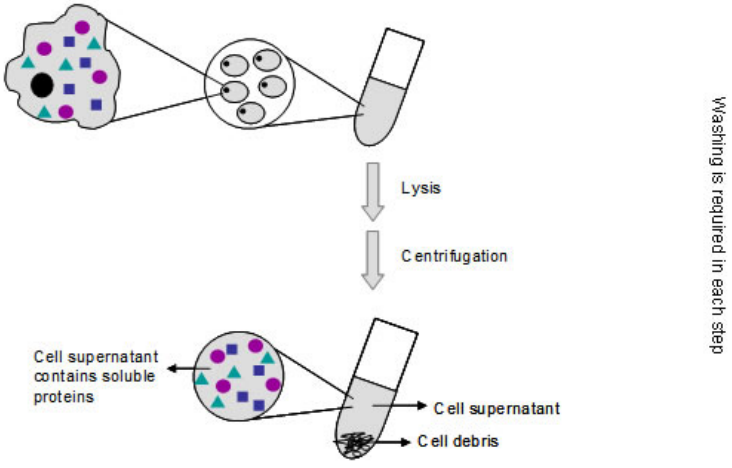
Immunoprecipitation (IP)

IP theory overview

Immunoprecipitation (IP) enables the purification of a protein (an antigen) from a mixture, taking advantage of the natural antibody/antigen affinity. This method not only allows the concentration of proteins of interest that would otherwise be difficult to detect in applications such as western blotting. IP can also help identify protein-protein, protein-DNA or protein-RNA interactions by immunoprecipitating their complexes. Basically, a protein mixture or cell lysate containing the protein of interest is incubated with a specific antibody solution. The antibodies will recognize and specifically bind to the corresponding antigens (proteins of interest). Antibodies are either previously immobilized on a solid-phase support (e.g. super-paramagnetic beads or non-magnetic agarose beads) or will be bound to protein A or G which has been immobilized on a solid-phase support. The solid support is sedimentable and thus indirectly precipitates the antibody/antigen complexes. Antigens can then be retrieved by dissociating the complexes.

Types of immunoprecipitation

There are 4 main types of immunoprecipitation. Each serves its own purpose but shares the same principle, as described above. 1. Individual protein immunoprecipitation (IP) This serves as a general method in immunoprecipitation and involves using an antibody to isolate and purify a particular protein of interest from a mixture such as cell or tissue lysate. 2. Co-immunoprecipitation (Co-IP) Co-immunoprecipitation, also known as protein-complex immunoprecipitation, serves as a powerful technique in the analysis of protein-protein interactions. It uses an antibody to target a specific protein which is a member of a protein complex. In this way, it is possible to isolate the entire protein complex out of the mixture and thereby identify unknown members of the complex. 3. Chromatin immunoprecipitation (ChIP) Similar to co-immunoprecipitation, but rather than studying unknown members of a protein complex, chromatin immunoprecipitation enables the study of protein-DNA interactions. ChIP is used to determine the location of DNA binding sites on the genome for a given protein of interest. This method relies on the formation of cross-links between primary amines located on amino acids and the bases on DNA molecules using formaldehyde. Following the cross-linking, the chromatin is sheared into small DNA fragments. A specific antibody is then used to precipitate the protein-DNA complex against the protein of interest. Since the cross-links are reversible, the DNA fragments of interest can be dissociated from the binding proteins by heating and these binding proteins can then be digested by proteases. The location that the specific protein binds to, can then be determined by PCR. 4. RNA immunoprecipitation (RIP) In contrast to chromatin immunoprecipitation, which targets DNA binding proteins, RNA immunoprecipitation targets RNA binding proteins. RNA bound by a specific protein can be identified by RT-PCR (reverse transcription-PCR) from the purified RNA in protein-RNA complexes. In addition to the immunoprecipitation methods mentioned above, researchers sometimes fuse tags such as Glutathione-S-Transferase (GST) onto either the C-terminal or N-terminal of the protein of interest. This approach allows the same tag to be used for different proteins and eliminates the difficulty of generating different antibodies each time.



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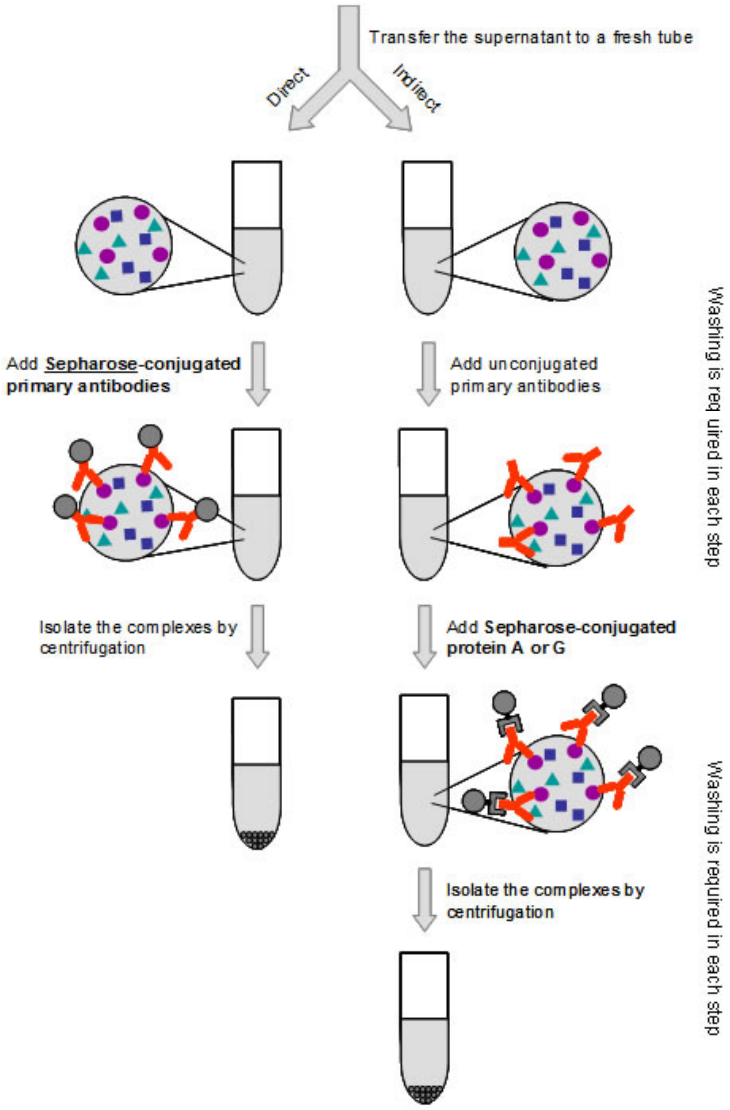

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