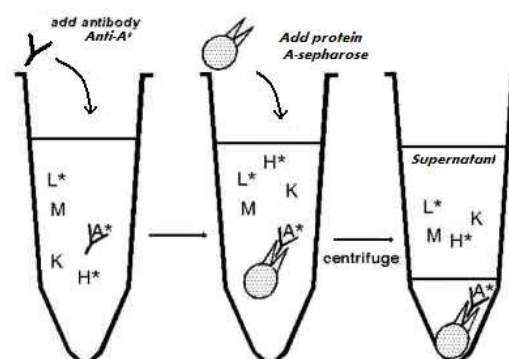


IMMUNOPRECIPITATION

Introduction

Immunoprecipitation (IP) is the technique of precipitating an antigen out of solution using an antibody specific to that antigen and to study protein-protein functional interactions. This process can be used to enrich a given protein to some degree of purity or to concentrate a low-abundance protein.

An antibody for the protein of interest is incubated with sample lysate (for instance cell extract or plasma) so that the antibody will bind the protein in solution. The antibody/antigen complex will be pulled out of the sample using protein A/G-coupled agarose beads. This physically isolates the protein of interest from the remaining proteins. The sample can subsequently be separated by SDS-PAGE for further determination (for instance Western Blot analysis).



Materials / reagents

- **Sample**
- **Antibody**
- **Pre-immune or non-immune serum**
- **Beads (protein A-sepharose or G-sepharose)**
- **Phosphate Buffered Saline (PBS)**
- **Lysis buffer:**
 - 0.5M Tris-HCl, pH 7.4
 - 1.5M sodium chloride
 - 2.5% deoxycholic acid
 - 10% NP-40
 - 10mM EDTA
 - Buffer can be stored at room temperature for six months.
- **Loading Buffer;** loading/sample buffer used for Western Blotting:
 - 125 mM Tris pH 6.8
 - 4% SDS
 - 10% glycerol
 - 0.006% bromophenol blue
 - 1.8% β-mercaptoethanol.

Procedure

If using a polyclonal antibody choose protein A-coupled sepharose beads. If using a monoclonal antibody choose protein G-coupled sepharose beads.

Lyse cells

An example of how to prepare cell lysate

1. Rinse the cells 2 times with PBS.
2. Add 1 ml of **lysis buffer** (pre-chilled), shake for 10 minutes at 4°C.
3. Collect the cells in microcentrifuge tubes.
4. Spin the samples at 14,000 rpm, 15 minutes, 4°C.
5. Transfer the supernatant to a new tube.

Pre-clearing (protocol toevoegen)

Pre-clearing helps to decrease the background and remove non-specific proteins that bind to protein A or G beads. This step is optional but required when working with material that has a lot of unrequired protein A or G-binding proteins.

6. Pre-clear the sample using pre-immune or non-immune serum.
- 7.

Prepare sample for immunoprecipitation

8. Add 5-10µg of antibody to the tube containing the cold precleared lysate on ice and allow antibody-antigen complex formation. (antibody can be labeled for later detection).
9. Incubate at 4°C for 1 hour to overnight (depending on the amount of protein and affinity properties of the antibody), preferably under agitation.

Adding the beads

10. Preparation following manufacturers instruction.

Pre-swollen beads as slurry which are ready for use are also available.

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The information on this sheet should neither be considered comprehensive or definitive.

11. Mix the slurry well and add the amount of beads according to the manufacturers (shake to suspend slurry before pipetting) to each sample. Always keep samples on ice. Beads will tend to stick to the sides of the tip so try to minimize the movement in the pipette and use a tip cut 5 mm from the top.
12. Incubate the lysate-beads mixture at 4°C under rotary agitation for 4 hours (the optimal incubation time can be determined in a preliminary experiment).

Precipitate the complex of interest, removing it from bulk solution.

13. When the incubation time is over, centrifuge the tubes.
14. Remove the supernatant and wash the beads in lysis buffer three times, centrifuge each time at 4°C and remove the supernatant.
15. After final wash, remove as much supernatant as possible (this step is most efficient when performed using spin columns so the entire volume of buffer can be removed at each wash step).

Elute proteins from solid support.

16. After removing the last supernatant add [loading buffer](#).
17. Boil at 95-100°C for 5 minutes to denature the protein and separate it from the protein-A/G beads.
18. Centrifuge and keep the protein containing supernatant.
19. The samples can be frozen or the complexes or antigens of interest can be analyzed. This can be done in a variety of ways, like identifying them on a SDS-PAGE, (expose the film if you used radioactivity), or with a Western Blot.

Safety

- Under no circumstances shall Hbt be liable for any damage arising from the use of this protocol. User should be trained and be familiar with the test procedure.
- Samples of tissue, serum or blood origin should be handled to guidelines for prevention of transmission of blood borne diseases.
- Some enzyme substrates are considered hazardous, due to potential carcinogenicity. Handle with care and refer to Material Safety Data Sheets for proper handling precautions.
- Wear appropriate protective clothing, gloves, and eyewear to avoid any accidental contact with reagents.
- Use extreme caution, is a known carcinogen.
- Reagents which contain preservatives may be toxic if ingested, inhaled, or in contact with skin.

Notes

- Protocol is to be used for research purposes only.
- Please note that this is a general protocol. Optimal dilutions for the antibody, cells preparation, controls, as well as incubation times will need to be determined empirically and may require extensive titration. Ideally, one would use the primary antibody as recommended in the product data sheet.
- When making buffer(s) fresh every time, there is no need to add sodium azide to the buffer.
- Sodium azide is an inhibitor of horseradish peroxidase (HRP). Do not include sodium azide in buffers or wash solutions if an HRP-labeled antibody/conjugate will be used for detection.
- The appropriate negative and positive controls should be included in every trial.
- When To determine radio labeled protein, one must remove unincorporated radiolabel first. This can be done by dialysis or gel filtration. Alternatively, incorporation of radiolabel can be determined by trichloroacetic acid precipitation.
- Note that Tris buffers are very temperature-sensitive and should be prepared using water at the temperature they will be used. Other buffers such as phosphate can be used instead of Tris.
- SDS tends to precipitate at 4°C although low concentrations used here may not be a problem; the SDS can be replaced with an equal amount of sarkosyl if is desired to store the buffer in the refrigerator.

Technical assistance

For technical assistance and more information please contact Hycult Biotech directly at support@hycultbiotech.com