Protocol for Immunoprecipitation (Co-IP)

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Abstract

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Protocol

Step 1.

Co-Immunoprecipitation, a classical method for studying protein-protein interaction, is a subcategory of immunoprecipitation. It is often utilized to identify unknown protein components in specific protein complexes. The Co-immunoprecipitation is based on the idea that if a know protein is a member of a large protein complex, then the whole protein complex may be “pulled” (often known as pull down)from the solution using its specific antibodies, and can be used to identify other unknown members of this complex. The characteristics of immunoprecipitation can be summarized into two points: the first is the natural state and the second the protein complex.

Advantages

Compared with other methods studying protein-protein interaction (such as GST-pull down, yeast two-hybrid), the protein interaction identified by immunoprecipitation is occurred in the cell and avoided human influence. Then it’s more close to the actual situation and the protein got is more reliable.

Operating methods

1. Wash cultured cells with pre-chilled PBS for 2 times carefully
2. Add in cold RIPA lysis buffer
3. Scrap cells off to clean 1.5ml eppendorf tubes with a clean, cold scraper. Put them on a low-speed rotating shaker for 15 min at 4°C
4. Centrifuge at 14,000 g 4°C for 15min, then transfer the supernatant to new tubes immediately
5. Wash protein A/G-agarose beads for 2 times with PBS and make a 50% protein A/G agarose working solution (in PBS)
6. Add in 50% protein A/G agarose with ratio of 100µl for a 1ml sample solution. Shake on horizontal shaker for 10minat 4°C (This step aims to eliminate non-specific binding proteins)
7. Centrifuge 14,000g at 4°C for 15min, then transfer the supernatant to new tubes and discard protein A/G-agraose beads
8. Quantify total protein with BCA assay or other methods
9. Dilute the total protein to 1µg/µl with PBS to decline the concentrations of detergents. If you

feel the concentration of your target protein is low, you can dilute the total protein to 10µg/µl. (if it's high enough)

10. Add in appropriate amount of primary antibody to approximately 500µl total volume
11. Slowly shake antigen-antibody complex on rotating shaker at 4°C overnight
12. Centrifuge 14,000g for 5s, and keep the pellet and wash with pre-chilled washing buffer (or cold PBS) for 3 times (800µl each)
13. Collect the supernatant to proceed to SDS-PAGE, western-blot, or mass spectra analysis