Immunoprecipitation for Cell Culture

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[Abstract] Immunoprecipitation (IP) is a method to pull down a protein out of solution using an antibody that specifically binds to that particular protein. Immunoprecipitation is a powerful technique to isolate and concentrate a particular protein from a sample containing many thousands of different proteins, to test protein-protein interactions, and to pull multiple members of a complex out of solution by latching onto one member with an antibody. This protocol describes a general immunoprecipitation strategy using cell cultures as starting material.

Materials and Reagents

1. HeLa S3 and HeLa cells were cultured in Dulbecco's modified Eagles Medium containing 10% fetal bovine serum (Invitrogen) and antibiotics
2. Protein A beads (Bio-Rad Laboratories); Protein G beads (Santa Cruz Biotechnology)
   Note: For rabbit polyclonal antibodies and mouse monoclonal antibodies IgG2a, IgG2b, IgG3, we usually couple antibodies to protein A beads; For mouse monoclonal antibodies IgG1, rat monoclonal antibodies, and mouse, rat, goat, and donkey polyclonal antibodies, we usually couple antibodies to protein G beads.
3. Bifunctional cross-linker dimethyl pimelimidate (DMP) (MW: 259.177) (Sigma-Aldrich, catalog number: D8388)
4. Phosphate buffered saline (PBS)
5. Tween 20
6. Triton X-100
7. Sodium borate (pH 9.0)
8. Hepes
9. KCl
10. NaN_3
11. NP-40
12. Glycerol
13. EGTA
14. $MgCl_2$
15. DTT

16. Microcystin
17. Leupeptin
18. Pepstatin
19. Chymostatin
20. β-mercaptoethanol
21. Bromophenol blue
22. PBST buffer (see Recipes)
23. Lysis buffer (see Recipes)
24. 1x SDS protein gel sample loading buffer (see Recipes)

Equipment

1. Hematology/chemistry mixer (Fisher Scientific)
2. Centrifuge (Eppendorf 5415D centrifuge)
3. Incubator

Procedure

A. Coupling antibody to Protein A beads

1. Wash 30 µl beads with 20 fold volume (20 vol) PBS or PBST twice.
   Notes:
   a. *For washing beads, we usually add buffer to the beads, mix in the tube several times, spin down the beads, and remove the supernatant.*
   b. *Spin down the beads at 4,000 rpm, 30 sec.*
   c. *We usually couple 1 µg antibody with 3 µl beads. If beads are stored in 1: 1 storing buffer, thus take 6 µl of the suspension of beads and buffer.*
2. Dilute 10 µg antibody in 100 µl PBS.
3. Add diluted antibody to beads, rotate on the Hematology/chemistry mixer equipment for 1 h at room temperature (RT).
4. Spin down beads, wash and resuspend in 20 vol 0.2 M sodium borate (pH 9.0).
5. Add 20 mM DMP to crosslink the antibody to the beads.
   Note: We usually take dry DMP powder directly (DMP powder is stored at 4 °C) and add 5.2 mg DMP per 1 ml total sodium borate suspension.
6. Incubate DMP in the sodium borate for 30 min, rotate, RT.
7. Spin down the bead and wash once with 20 vol 1 M Tris-HCl (pH 7.7).
8. Spin down the bead and resuspend in 20 vol 1 M Tris-HCl (pH 7.7). Incubate for 2 h, rotate, RT, to quench the activity of DMP.

9. Spin down beads and wash with PBS twice.
10. Spin down the beads and store antibody coupled beads in 60 µl PBS (33% beads), add 0.05% NaN_3. Store the beads at 4 °C for a couple of months.

B. Lysis of cells

11. Lyse the cells in 7 vol lysis buffer.
12. Put the lysis solution on ice for 30 min to 1 h.
13. Centrifuge the lysis solution at 13,000 rpm for 10 min, 4 °C.
14. Transfer supernatants. The supernatants can be stores at -80 °C for future use.

C. Coimmunoprecipitation

15. Incubate 3 µl antibody coupled beads in 250 µl cell lysis supernatants, rotate overnight at 4 °C.
16. Wash beads 4 times with 20 vol lysis buffer containing 500 mM KCl and 0.5 % NP-40, and wash once with lysis buffer.
    Note: For wash beads, we usually add buffer to the beads, mix the tube several times, spin down the beads, and remove the supernatant.
17. Elute protein with 1x SDS protein gel sample loading buffer, separated by SDS-PAGE (5-15%) and analyze by western blot.

Recipes

1. PBST buffer
   PBS plus 0.1 % Tween 20 or 0.1 % Triton X-100
2. Lysis buffer
   50 mM Hepes (pH 7.4)
   200 mM KCl
   0.3% NP-40
   10% glycerol
   1 mM EGTA
   1 mM $MgCl_2$
   0.5 mM DTT
   0.5 µM microcystin
   10 µg/ml each of leupeptin, pepstatin, and chymostatin
3. 1x SDS protein gel sample loading buffer
   50 mM Tris-HCl (pH 6.8)
   2% SDS

10% glycerol
1% β-mercaptoethanol
12.5 mM EDTA
0.02 % bromophenol blue

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References

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