# 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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