

## 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<sub>3</sub>
11. NP-40
12. Glycerol
13. EGTA
14. MgCl<sub>2</sub>
15. DTT

16. Microcystin
17. Leupeptin
18. Pepstatin
19. Chymostatin
20.  $\beta$ -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  $\mu$ l 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  $\mu$ g antibody with 3  $\mu$ l beads. If beads are stored in 1: 1 storing buffer, thus take 6  $\mu$ l of the suspension of beads and buffer.*

2. Dilute 10  $\mu$ g antibody in 100  $\mu$ l 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  $\mu$ l PBS (33% beads), add 0.05%  $\text{NaN}_3$ . Store the beads at 4  $^{\circ}\text{C}$  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  $^{\circ}\text{C}$ .
14. Transfer supernatants. The supernatants can be stores at -80  $^{\circ}\text{C}$  for future use.

#### C. Coimmunoprecipitation

15. Incubate 3  $\mu$ l antibody coupled beads in 250  $\mu$ l cell lysis supernatants, rotate overnight at 4  $^{\circ}\text{C}$ .
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  $\text{MgCl}_2$   
0.5 mM DTT  
0.5  $\mu$ M microcystin  
10  $\mu$ g/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%  $\beta$ -mercaptoethanol  
12.5 mM EDTA  
0.02 % bromophenol blue

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### **References**

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