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Co-immunoprecipitation in human cells

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ABSTRACT

Protocol to perform co-immunoprecipitation (co-IP) in human cells. If the co-IP is followed by proteomics, it is better to remove the glycerol from the different buffers.

PROTOCOL CITATION

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Day 1 10m

1 15m

1. Split human cells in a 150 mm dish to obtain a ~80% confluence on Day 2.
2. Preblock Dynabeads protein A and/or G or Dynabeads M-280 Sheep anti-mouse IgG with 1 ml 0.5% BSA in PBS overnight on a rotating wheel at 16 rpm at 4°C.

For Dynabeads protein A and G (30 mg/ml): use 40 µl of beads per condition (at least two: one IgG control and one IP).
For Dynabeads M-280 Sheep anti-mouse IgG (10 mg/ml), use 120 µl of beads per condition.

Day 2 4h 35m

2 10m

Beads preparation

1. Wash the beads three times on ice with a magnetic rack with 600 µl of IP buffer (25 mM Tri–HCl pH 8.0, 150 mM NaCl, 0.5% NP-40, 10% Glycerol, 2.5 mM MgCl2, and protease inhibitor cocktail). Perform the washes by gently mixing by inversion.
2. Add 2-5 µg of antibody to the beads in 600 µl IP Buffer.
3. Incubate for at least 2 hours on a rotating wheel at 16 rpm at 4°C.

2.1 1h 20m

Lysis

1. Cells should be ~80% confluent.
2. Prepare 8 ml of ice-cold PBS with protease inhibitor cocktail per condition.
3. Wash cells twice with ice-cold PBS.

4. Scrap the cells in 8 ml of ice-cold PBS prepared in step 2 of 2.1 and transfer to an ice-cold 15 ml Falcon tube.
5. Centrifuge at 500 g for 5 minutes at 4°C.
6. Remove the supernatant and resuspend the pellet in 800 µl Lysis Buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% NP-40, 10% glycerol, 2.5 mM MgCl2, protease inhibitor cocktail, phosphatase inhibitor, 1× PMSF, and 25–29 units of Benzonase) by pipetting up and down ~ 10 times and transfer in an ice-cold 1.5 ml tube.
7. Incubate on a rotating wheel at 16 rpm for 30minutes at 4°C.
8. Centrifuge at 13,000 g for 15 minutes at 4°C.
9. Transfer the supernatant in a new ice-cold 1.5 ml tube. Add 800 µl of Dilution Buffer (150 mM NaCl, 10% glycerol, 2.5 mM MgCl2, protease inhibitor cocktail, phosphatase inhibitor, and 1× PMSF) and mix by pipetting up and down ~ 8-10 times.
10. Perform Bradford or another protein quantification method to determine the concentration of each sample.
11. Freeze at -20°C at least 100 µl of sample to be used as Input.

2.2 IP

2h 30m

1. Wash the beads three times on ice with a magnetic rack with 600 µl of IP buffer.
2. Add 1 mg of proteins to the 1.5 ml tubes containing the washed Ab-conjugated beads (top to 600 µl with IP buffer).
3. Incubate on rotating wheel at 16 rpm for 2 hours in a cold room.
4. Wash the beads three times on ice with a magnetic rack with 600 µl of IP buffer (first wash: 5 minutes incubation on a rotating wheel at 16 rpm; no incubation for the second and third washes).
5. Wash the beads three times on ice with a magnetic rack with 600 µl of IP buffer without NP-40 (first wash: 5 minutes incubation on a rotating wheel at 16 rpm; no incubation for the second and third washes).
6. Do a quick spin down (3 seconds at 800 rpm) and put back the tubes on the magnetic racks to remove the last drops.
7. The beads can be freeze at -20°C to perform protein digestion on beads for proteomics or to perform later the elution.

2.3 Elution with LDS for western blots

15m

1. Add 36 µl 1x LDS + 4 µl DTT (1M stock solution) to each tube, and resuspend the beads by vortexing with a low strength.
2. Incubate on a Thermomixer for 10 minutes at 70°C at 1,400 prm.
3. Perform a quick spin and put the tube on a magnetic rack.
4. Transfer the supernatant to a fresh protein Lobind 1.5 ml tube. Use 20 µl per western blot (one elution = two western blots).
5. Store at -20°C. Add DTT again before loading on a gel (2 µl per 20 µl sample).

2.4 Elution with glycine for proteomics

20m

1. Add 1.5x volume of 0.2M glycine solution at pH 2.3 to the beads. Resuspend the beads by pipetting up and down.
2. Incubate on a Thermomixer for 7 minutes at 25°C at 1,000 rpm.
3. Perform a quick spin and put the tube on a magnetic rack.
4. Transfer the supernatant to a fresh protein Lobind 1.5 ml tube.
5. Neutralise by adding $1/10^{th}$ of the volume of 1M Tris-HCl pH 8.5.
6. Repeat the glycine elution once (steps 1 to 5, part 2.4).
7. Pool both fractions in the same tube.