MCPyV Co-Immunoprecipitation Protocol

Version 2

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PROTOCOL STATUS

Working

MATERIALS TEXT

Nuclear Extract Buffer A

ddH_2O	--> 29.24mL	
1M HEPES pH 7.9	--> 300ul	Final Concentration: 10mM
3M KCl	--> 100uL	Final Concentration: 10mM
0.1M EDTA	--> 30uL	Final Concentration: 0.1mM
0.1M EGTA	--> 30uL	Final Concentration: 0.1mM
0.1M DTT	--> 300uL	Final Concentration 1mM

Protease Inhitibtor

Final Volume: 30mL

150mM KCl Buffer

ddH_2O	--> 14.3mL	
1M TrisCl pH 8.0	--> 400uL	Final Concentration: 20mM
50% Glycerol	--> 4mL	Final Concentration: 10%
10% Tween-20	--> 200uL	Final Concentration: 0.1%
1M $MgCl_2$	--> 100uL	Final Concentration: 5mM
3M KCl	--> 1mL	Final Concentration: 150mM

Protease Inhibitor

Final Volume: 20mL

10% NP40

ddH_2O	--> 9mL
NP40	--> 1mL

1% BSA in PBS

PBS	--> 10mL
BSA	--> 0.1g

Day 1 - Plate Cells

1 The night before transfection, seed a 10cm plate with enough cells to be ~80% confluent the following afternoon.

Day 2 - Transfection

2 Transfect the cells using your preferred transfection reagent/protocol.

Day 4 - Harvest/Lysis/IP Part 1

3 **HARVEST/LYSIS**

36-48 hours post transfection harvest the cells.

1. Wash the cells with cold PBS.
2. Scrape the cells into cold PBS (10mL). Add PBS cell suspension to 10mL conical tube.
3. Spin tubes at 300g, 4.C, for 5 min.
4. Working on ice, suction off PBS.
5. Resuspend cellular pellets in 533uL of Buffer A.
6. Add 33uL of 10% NP40. Pipette well. Do not vortex.
7. Let tubes sit on ice for 5 minutes.
8. Sonicate samples in ice at 20% amplitude for 8 seconds, with 1 second pulse on, 1 second pulse off.
9. Rotate lysates in cold room for 1 hour.
10. Spin lysates at 14000rpm, 4.C, 15min.
11. Transfer cleared lysates to prechilled eppendorf tubes.

Determine Protein Concentration

1. Perform BCA assay (or preferred method) to determine protein concentration.
2. For cellular lysate, add 30ug of cleared cellular lysate to SDS sample buffer. Boil at 95.C for 5 minutes.
3. Cool the samples on ice, centrifuge.
4. Either save at -20.C till the entire IP is complete (tomorrow), or run 30ug of each sample in an 8-16% Tris-Glycine gel at 150V for 50 minutes.

IP Day 1

1. For each sample, prepare 2 tubes containing 25uL of A/G magnetic beads + 500uL of cold PBS. (For example, if you have 10 samples, add 500uL of beads to 10mL of cold PBS. Mix. Add 525uL of the bead/PBS mixture into each of the 20 tubes.)
2. Place the tubes in a magnetic rack, remove the PBS.
3. Add 500uL of PBS to each tube of beads. Resuspend the beads.
4. Place the tubes in a magnetic rack, remove the PBS.
5. Add 500ul of KCl buffer to each of the tubes. Resuspend the beads.
6. Place the tubes in a magnetic rack, remove the KCl buffer.
7. For one set of 10 tubes, block the beads. Add 500uL of 1% BSA. Rotate these tubes in the cold room, overnight.
8. With the second set of tubes, pre-clear the lysates. Add 500ug of lysate to the 25uL of washed beads. Add Buffer A until the final volume reaches ~500uL. Rotate in cold room for 1 hour. Remaining lysate may be kept at -80.C.
9. After 1 hour, do a short spin on the pre-cleared lysates. Place the tubes in a magnetic rack. Transfer the lysates to a clean tube. Discard the beads.
10. Add 10ug of the antibody used in the immunoprecipitation to the pre-cleared lysates. Rotate in cold room, overnight.

Day 5 - IP Part 2

4 **IP Day 2**

1. Obtain both sets of tubes from the cold room (blocked beads and lysate/antibody samples).
2. Remove the BSA from the beads using a magnetic rack.
3. After a short spin, add the lysate/antibody samples to the beads.
4. Rotate for 1 hour at room temperature.
5. After a short spin, remove the lysate from the beads.
6. Wash several times with KCl buffer.
7. After removing the KCl buffer from the final wash, resuspend the beads in 30uL of 2X SDS sample buffer.
8. Boil at 95.C for 5 minutes. If not running a gel immediately, store each sample at -20.C.
9. Run each sample in an 8-16% Tris-Glycine gel at 150V for 50 minutes.
10. Transfer each gel to a Immobilon-P membrane.
11. Block, immunoblot, and develop with desired antibodies.