Protocol: Immunoprecipitation

Kit: Pierce Classic IP Kit

Additional materials:

1. For cell culture
2. 0.1M Na_2HPO_4, 0.15M $NaCl_2$, Ph 7.2
3. Antibody: anti-AGO1 (clone 4B8, Sigma) and anti-AGO2 (clone 11A9, Sigma) produced in rat

Note:

1. All steps have to be carried on ice or at 4°C.
2. Centrifugation at 1000g for 30s to 1 min at 4°C.

Procedure

1. Plate cells in a 25cm2 flask, and incubate until 80-95% confluence is reached
2. Trypsinize and collect cells, wash with PBS, transfer to microcentrifuge tube
3. Add 1.5 ml ice cold IP Lysis/Wash Buffer to the cells. Incubate on ice for 5 minutes with periodic mixing.
4. Transfer the lysate to a microcentrifuge tube and centrifuge at ~ 13,000 xg for 10 minutes to pellet the cell debris. Transfer supernatant to a new tube for protein concentration determination and further analysis. Measure protein concentration using Bradford reagent.
5. Pre-clear lysate using the Control Agarose Resin
6. 1. For 1mg of lysate, add 80µl Control Agarose Resin slurry (flick and mix thoroughly) into a spin column. Centrifuge column to remove storage buffer.
7. Add 100µl of 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 to the column, centrifuge and discard the flow-through. Insert the bottom plug into the column.
8. Add 1mg of lysate to the column containing the resin and incubate at 4ºC for 30 minutes to 1 hour with gentle end-over-end mixing.
9. Centrifuge column at 1000 ×g for 1 minute. Discard the column containing the resin and save the flow-through, which will be added to the immobilized antibody.
10. Combine 3-5µg of affinity purified antibody with the 600µg of pre-cleared cell lysate in a microcentrifuge tube.
11. Dilute the antibody/lysate solution to 300-600µl with IP Lysis/Wash Buffer.
12. Incubate overnight at 4ºC to form the immune complex.
13. Gently swirl the bottle of Pierce Protein A/G Agarose to obtain an even suspension. Using a wide-bore or cut pipette tip, add 20µl of the resin slurry into a Pierce Spin Column. Place column into a microcentrifuge tube and centrifuge at 1000u g for 1 minute. Discard the flow-through.
14. Wash resin twice with 100µl of cold IP Lysis/Wash Buffer.
15. Gently tap the bottom of the spin column on a paper towel to remove excess liquid and insert the bottom plug.

16. Add the antibody/lysate sample to Protein A/G Plus Agarose in the spin column. Attach the screw cap and incubate with gentle end-over-end mixing for 1 hour.
17. Remove bottom plug, loosen the screw cap and place the column in a collection tube. Centrifuge column and save the flow-through. Do not discard flow-through until confirming that the IP was successful.
18. Remove the screw cap, place the column into a new collection tube, and wash with IP Lysis buffer four times (each time with 200µl).
19. Wash the resin once with 100µl of 1X Conditioning Buffer (diluted in PBS).