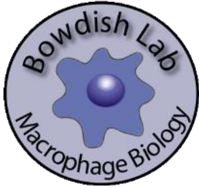

CO-IMMUNOPRECIPITATION

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EQUIPMENT AND MATERIAL

- Cells cultured in 10cm dishes, transfected with appropriate vectors, if needed
- PBS
- Lysis buffer (I use 1% Triton X-100 for scavenger receptors)
- Protein A/G/L-sepharose slurry
- Antibody used for pull-down (ideally will be a monoclonal antibody with high affinity)
- Cell scrapers
- Rocker

PROTOCOL

Co-immunoprecipitation:

1. Seed 10cm dishes at an appropriate density of cells for transfection (2×10^6 for transfection after 24h or 1×10^6 for transfection after 48h).
2. Transfect cells as outlined in the **PEI Transfection** protocol.
3. At 48h post-transfection, scrape cells off bottom of the dish into the growth media.
4. Spin down cells at 1,500rpm for 5min.
5. Remove supernatant and wash cell pellet with 10ml PBS.
6. Centrifuge as above.
7. Remove supernatant and re-suspend cells in 1ml of lysis buffer.
    a. Ensure that the lysis buffer contains protease inhibitors (5ul of Sigma protease inhibitor cocktail for mammalian cells for 1ml of buffer).
8. Incubate on ice for 10min.
9. Transfer lysates to a 1.5ml tube and centrifuge at 14,000rpm for 10min at 4°C.
10. Transfer supernatant into a clean 1.5ml tube. Save 50ul as an input control.
11. To the remaining supernatant, add 2μg of antibody used for immunoprecipitation and 125μl of Protein A/G/L-sepharose slurry.
12. Incubate rocking overnight at 4°C.
13. Pellet beads by centrifuging at 14,000rpm for 5min.
14. Wash beads with 1ml of lysis buffer 3×.
    a. Ensure that the lysis buffer contains protease inhibitors (5ul of Sigma protease inhibitor cocktail for mammalian cells for 1ml of buffer).
15. Completely remove supernatant.
16. Re-suspend beads in 30ul lysis buffer. Add 10ul of 4× SDS loading buffer. Boil 10min.
17. Can use immediately for Western blot analysis or be stored at -80°C.

Preparation of Protein A/G/L-sepharose slurry:

1. Dissolve 0.75g of Protein A/G/L-sepharose powder in 45ml of E1A buffer (0.1% NP-40, 50mM HEPES pH 7.0, 250mM NaCl in dH_2O).
2. Incubate 30min at 4°C rocking.
3. Pellet beads by centrifuging for 10min at 4,000rpm.
4. Wash beads 2× with 45ml of E1A buffer.
5. Re-suspend beads in 25ml E1A containing 0.01% sodium azide.
6. Store at 4°C.