Lazzara Lab
Chris Furcht
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Immunoprecipitation protocol

1. Add 250 µL 1X PBS to an eppendorf tube for each immunoprecipitation to be done.
2. Add 10 µL suspended protein-A (for rabbit capture antibodies) or protein-G (for mouse capture antibodies) beads to each tube with PBS. For pipetting beads, invert tube repeatedly until beads are in solution, then pipet up and down with a red pipette tip that is cut off to the first digit to make extra sure beads are well mixed before pipetting up. Repeat the same process every time you pull up beads; sometimes if beads are settled before pipetting the pipet tip will clog with beads.
3. Spin tubes at 4000 rpm for 3.5 min.
4. Remove supernatant with blue or green pipette tip, and volume up beads in 0.5 mL 1X PBS.
5. Add capture ab to tubes (for example, 500ug of total protein from H1666 cells needs 2 µL sc-280 antibody for an SHP2 IP). Allow the ab to equilibrate with beads by inversion rotation in cold room for 4 hr (or overnight). Make sure that beads in are solution and not pelleted during inversion.
6. After ab equilibration with beads, spin down (4000 rpm, 3.5 min) and aspirate supernatant. Wash beads once in 0.25 mL PBS, and spin at 4000 rpm for 3.5 min. Aspirate again, and then wash beads once in 0.25 mL 1X IP lysis buffer.
7. Thaw lysates and reserve some of the original lysate if desired (for blotting comparison to determine how much protein was captured).
8. Incubate 500 µg (or however much you want) lysate with beads. Volume up with lysis buffer if necessary to get 500 µL per tube. Equilibrate with inversion rotation in cold room overnight (not for too long though, >12 hr but <18 hr is ideal).
9. Spin down beads and reserve supernatant if desired.
10. Aspirate and wash beads 3X with 0.25 mL IP LB per tube, spinning at 4000 rpm for 3.5 min each time. After final wash, I use a green pipette tip followed by a red pipette tip to remove as much LB as possible.
11. Reconstitute beads in 500uL (or less if desired, but remember to adjust loading for blot to compare supernatant) of 1X SB (reconstitute with PBS) as lysate used and boil. Alternatively, for concentrating protein (probably what you want to do after optimizing and necessary for co-IP), reconstitute in 27 µL 1.5X SB and add 3 µL DTT as well. Boil for 10 min to remove protein from beads. Then proceed to standard western blotting protocol.

IP optimizations previously done:
H1666 SHP2: 10µL Protein A beads (sc-2001) + 2 µL SHP2 antibody (sc-280), 500 µg protein
U87MG M (1.5×10^6 EGFR/cell) EGFR: 15 µL Protein A beads + EGFR Ab (sc-03), 250 µg protein
U87MG H (1.5×10^6 EGFR/cell) EGFR: Need >15 µL Protein A beads + EGFR Ab (sc-03) for 200 µg protein
HeLa SHP2: 10 µL Protein A beads (sc-2001) + 2 µL Shp2 antibody (sc-280), 500 µg protein
U87MG SHP2: 10 µL Protein A beads (sc-2001) + 2.5 µL SHP2 antibody, 500 µg protein
HeLa (15min 10ng/mL EGF) pEGFR: 10 µL Protein A beads (sc-2001) + 5 µL pEGFR Y1068 ab (Epitomics #1138), 200 µg protein
H1666 EGFR: 10 µL Protein G beads + 2 µL EGFR antibody (Ab12), 500 µg protein
HeLa EGFR: 10 µL Protein G beads + 2 µL EGFR antibody (Ab12), 500 µg protein
A431 EGFR: 10 µL Protein G beads + 2.25 µL EGFR antibody (Ab12), 250 µg protein