V5 Pulldown

Isolate protein:

For adherent cells in a 6-well plate:

1. Remove media with vacuum.
2. Wash cells 1x with 1x PBS
3. Lyse cells with 350 μL IP lysis buffer (137 mM NaCl, 10mM Tris pH 7.4, with 1% NP-40 and Sigma Protease Inhibitor Cocktail 1:100) using rubber policeman.
4. Transfer lysate to 1.5 mL eppendorf tube on ice.
5. Incubate on ice 30 min, vortexing thoroughly every 10 min.
6. Centrifuge at 14,000 rpm for 15 min, 4°C.
7. Transfer 60 μL of the supernatant to a fresh 1.5 mL eppendorf tube containing 60 μL 2x tris-tricine sample buffer. Boil lysate 5 min at 100°C, centrifuge 14,000 rpm 1 min and store lysate at -20°C.
8. Transfer 250 μL remaining lysate to a fresh 1.5 mL eppendorf tube and add 5 μL V5-agarose beads (purchased through Bethyl Labs cat#S190-119).
    i. Invert the V5-agarose beads to mix before pipetting.
    ii. When pipetting the V5-agarose beads, cut off the tip of the pipet tip to ensure transport of the beads.

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1. Incubate lysates containing V5-agarose beads with rotation for 1 hour at 4°C.
2. After incubation, quick spin the lysates for 15 sec (14,000 rpm, 4°C).
3. Pipet off supernatant being careful not to disturb bead pellet.
4. Wash 4x with 250 uL IP lysis buffer (with 1% NP-40 and Sigma Protease Inhibitor Cocktail 1:100), centrifuging 15 sec (14,000 rpm, 4°C) pipetting off supernatant in between washes.
5. After final wash, remove supernatant with pipet leaving behind approx. 20-30 μL of the supernatant.
6. Elute by adding 25 μL 2x tris-tricine sample buffer to each sample.
7. Boil 5 min. 100°C then centrifuge 14,,000 rpm for 1 min.
8. Store IP lysates at -20°C until use.