**Co-immunoprecipitation (co-IP)**

(Protocol was originally from Dr. Yang Li, looks similar to the CD co-IP protocol: https://www.creative-diagnostics.com/co-immunoprecipitation-co-ip-protocol.htm)

Prepare lysis buffer (0.5% NP-40)

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|  | Final concentration |
| NP-40 | 0.5% |
| Tris-HCl, pH7.4 | 50 mM |
| NaCl | 150 mM |

1. **Harvest cells.** SMCs were cultured in 10 cm dish in incubator. Take out, wash with PBS, and then remove PBS. Move to next step or store in -80.
2. **Lysis cells.** Use 0.5% NP-40 (ice cold) as lysis buffer, make sure protease inhibitor (PI, 100X) was added. 2mL lysis buffer for one 10 cm dish. Put on a rocker in 4 C and rock for 15 mins, and rotate the plate 90 degree and rock for another 15 mins to make sure all the cells been lysised completely.
3. **Collect cell lysis.** Transfer the lysis in a 2mL tube, centrifuge 13000 rpm at 4C for 15 mins. Take the supernatant, 80 uL as input, 1mL for incubation with antibody+beads, 1mL for incubation with beads only (IgG control).

For 80 uL input, add 20 uL 5X SDS loading buffer, boil at 99C for 10 min, put on ice.

1. **Incubate with antibody.** Add 8 uL antibodies per tube (1mL lysis). Rotate at 4C for 30 mins.
2. **Wash beads.** Take the DynaBeads ProteinG, mix well. Take 15 uL beads for each reaction tube, put on magnetic stand for 30-60S, remove the supernatant. Add 1mL 0.5% NP-40 as lysis buffer, put on magnetic stand for 30-60S, and remove the supernatant. Repeat 2 times. In the end, re-suspend the beads with 100 uL 0.5% NP-40 as lysis buffer per 15 uL beads.
3. **Incubate with beads**. Take the lysis (from step 3, IgG control) and lysis+antibodies (from step 4), add 100 uL washed beads per tube, and rotate at 4C for 2.5h.
4. **Collect IPed protein**. Take the rotating tube out of 4C, put on magnetic stand for 30-60S, and remove the supernatant. Add 1mL 0.5% NP-40 in the tube, put on magnetic stand for 30-60S, and remove the supernatant. Repeat 2 times. Add 100 uL 1X SDS loading buffer for each tube, mix well by vortex, boil at 99C for 10 mins. Together with input from step3, centrifuge for 5 min, move to western blotting or store at -20.

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