Immunoprecipitation

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Proceedure

You can find many different protocols for immunoprecipitation in the literature. The following is the one (with low strigency) that we frequently use in the lab. To increase stringency, you can increase the salt concentration and add triton X-100 or NP-40 in the binding or washing buffer. The actual concentration needs to be determined experimentally.

Extraction Buffer:

50mM HEPES (pH7.4), 137 mM NaCl, 10% Glycerol.
Add protease inhibitors fresh to the buffer just before the extraction.
Protease inhibitor final concentration:
Pepstatin A (1μg/ml), Leupeptin (1μg/ml), PMSF (1mM)

Stock kept in -20˚C:
Pepstatin A: 1mg/ml in methanol.
Leupeptin: 1mg/ml in H_2O.
PMSF: 100 mM in isopropanol.

1. Grind tissue (2-400mg) in liquid nitrogen with a mortar and pestle.

2. Suspend ground tissue in ice-cold extraction buffer containing protease inhibitors. Mix by gentle inversion. Let sit on ice for 5 min and then mix again.

3. Centrifuge cell homogenate at 12,000 rpm for 15 min at 4˚C.

4. Keep the supernatant, and centrifuge cell homogenate at 12,000 rpm for 15 min at 4˚C again.

5. Remove the supernatant carefully (avoid the lipids on top and pellet at bottom) and measure the protein concentration.
   Mix 2 μl protein extract with 800 μl H_2O and 200 μl Bio-Rad protein assay dye. Mix and measure OD_{595} and calculate the protein concentration.

6. Incubate 0.5-5 mg total extract diluted in 1ml IP buffer (extraction buffer without Glycerol) + Antibody (Ab concentration needs to be determined experimentally – 1:200 will be a good start for polyclonal antibody), rotate on rotator for 2~4 hrs at 4^{o}C.

7. Add pre-equilibrated (with IP buffer) protein G beads (GammaBind G sepharose (Amersham Biosciences, cat# 17-0885-01)15~20 μl (bead volume), rotate 1hr at 4^{o}C,.

8. Pellet the protein G beads with a brief spin at 4^{o}C, 4,000g X 1min, discard the supernatant carefully by gentle vacuum, then add 1ml IP buffer to resuspend and wash the pellet.

9. repeat step 8 3~4 times

*To decrease the background, you may change the tube during wash once or twice.

10. Add 70μl of 1X SDS loading buffer to resuspend the beads and boil for 5 min.

11. Take the supernatant after brief spin, load onto SDS-PAGE gel.

References: Way too many to reference a specific one.