

Co-immunoprecipitations

Take confluent dishes (10 cm or 15 cm) of T/C cells. Aspirate medium and quickly wash three times with PBS. Add 0.5 ml / 1 ml of cold IP buffer. Incubate on a rocker at 4°C for 10 mins.

Harvest cells by scraping. Collect and pool lysates, finally washing all the plates with a single 0.5 ml / 1 ml aliquot of IP buffer, adding this to the pool. Spin in a microfuge at 14,000 rpm for 5 mins. Remove s/n to a fresh tube.

Determine protein concentration of lysate using a Bradford assay. Typical protein concentration should be 1.0 – 1.5 mg/ml. 1 mg of lysate should be used per immunoprecipitation reaction.

Preclear lysates with 50 µl of 50% protein A-sepharose / protein G-agarose per 1 ml of lysate. Incubate on a tumbling wheel at 4°C for 1 hour. Pellet sepharose in a microfuge, and transfer lysate to a fresh tube.

For each reaction, add 5 – 10 µg of immunoprecipitating antibody. Incubate on a tumbling wheel at RT for 2 hrs (minimum), or preferably at 4°C overnight. Note : always prepare control reactions without the addition of antibody.

Add 50 µl of 50% protein A-sepharose / protein G-agarose. Incubate on a tumbling wheel at RT for 2 hours.

Pellet complex in a microfuge. Wash 5x with IP wash buffer, ensuring that as much liquid as possible is removed after the final wash. Finally add 15 µl of 3x reducing sample buffer, boil for 10 minutes, spin in microfuge for 5 minutes then load s/n on a mini-gel.

IP Buffer

150mM NaCl
1% NP40
50mM Tris-HCl, pH8.0
Complete PI*
0.1 mM PMSF*

IP Wash Buffer

PBS
0.1% NP40

* add fresh