

Standard immunoprecipitation protocol

1. Use 1 x 10 cm dish of near confluent cells for 1 ml of lysis buffer.
2. Wash cells in 2 ml ice cold PBS.
3. Lyse cells with 1 ml of 1x lysis buffer containing protease inhibitor cocktail (PIC). Wash cells off of plate using a pipette or cell scraper.
4. Centrifuge at 4°C for 15 mins at 15000 rpm to pellet insoluble material.
5. Carefully remove supernatant to a fresh tube.
6. Add 1 µg of primary antibody and incubate with gentle mixing for 2-16 hours at 4°C.
7. Wash 20 ml of Protein G-sepharose in 1ml of lysis buffer
8. Resuspend in 100 µl and add to lysate containing antibody.
9. Incubate 1 hour with gentle mixing at 4°C.
10. Wash complexes 3 times in lysis buffer + PIC at 4°C.
11. Boil in 50 µl SDS sample buffer prior to loading on gel
12. Run on SDS-PAGE and immunoblot.

Lysis buffer

Tris-HCl: 50 mM, pH 7.4

NP-40: 1%

Na-deoxycholate: 0.5%

NaCl: 150 mM

COMPLETE protease inhibitors (Roche)