

### Immunoprecipitation

1. Lyse cells with lysis buffer containing (in mM): 9.1 dibasic sodium phosphate, 1.7 monobasic sodium phosphate, pH 7.4, 150 NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 0.25 phenylmethylsulfonyl fluoride, 1 µg/ml aprotinin, 1.0 activated sodium orthovanadate, 5.0 sodium pyrophosphate for 1 hour at 4°C.
2. Centrifuge the lysate at 12,000 x g for 2 minutes at 4°C.
3. Transfer the supernatant into a fresh microtube and hold on ice.
4. Preclear the lysate using 10 µl of protein G-agarose conjugate (Sigma) and incubate for 1 hour at 4°C.
5. Centrifuge the mixture at 12,000 x g for 5 minutes at 4°C. Transfer the supernatant to a fresh microfuge tube.
6. Divide the supernatant into two aliquots and add sufficient NET gel buffer containing (in mM): 50 Tris-HCl, pH 7.5, 150 NaCl, 0.1% NP-40, 1.0 EDTA, pH 8.0, 0.25% gelatin, 0.02% sodium azide to obtain a volume of 0.5 ml for each aliquot.
7. To one aliquot of supernatant, add 1 µg of affinity-purified antibody against the target protein. To the second supernatant, add 1 µg of control antibody or preimmune serum. Incubate for 1 hour at 4°C.
8. Centrifuge the mixture at 12,000 x g for 5 minutes at 4°C.

9. Discard the supernatant and add one volume of RIPA buffer to resuspend the Protein G-agarose beads. Incubate for 30 minutes at 4°C. Centrifuge the mixture at 12,000 x g for 5 minutes at 4°C. Repeat 2 more times.

Add 20 µl of SDS sample buffer. Run on SDS-PAGE gel to analyze immunoprecipitated complex