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PRODUCT-SPECIFIC PROTOCOLS

IP (15222-1-AP)

Sample type	Amount of protein used	Amount of primary antibody	Incubation time	Beads used	Incubation time	Elution buffer	WB- Primary antibody	WB -Secondary antibody
NIH/3T3 cells	1600ug	4ug	12.0 h at 4.0°C	Protein A Sepharose beads	4 h at 4°C	0.10M Glycine, 500mM NaCl,0.05M Tris - HCl,(pH 1.5 - 2.5)	1:1000	

PROTOCOL

1. Prepare lysate using standard protocol.
2. Add primary antibody to the lysate and gently rock the sample mixture at 4°C for 2-4 h or overnight. Set up a negative control with control IgG corresponding to the primary antibody source.
3. Add sepharose bead slurry to capture the immunocomplex and gently rock the mixture for 4 h at 4°C.
4. Centrifuge the mixture at 500-1000 rpm (110-220x G's) for 30 sec at 4°C and discard the supernatant.
5. Wash the beads 3-4 times with 1 ml 0.2% TBST, centrifuge and discard the supernatant as in step 4
6. Elute the pellet twice with 40 µl elution buffer containing 500 mM NaCl. Pool elutions and then neutralize by adding 10 ul Alkali neutralization buffer containing 1 M NaOH (pH 6.8-7.2).
7. Add 30 ul 4X SDS sample buffer to the final elution. Heat at 95°C for 5 min.
8. Centrifuge 3 min at 11,000 rpm (10,000x G's). Load supernatant (20- 30% total volume) onto SDS-PAGE gel; alternatively, collect the supernatant carefully to a new tube and store at -80°C for later use.
9. Run the gel and apply protein transfer. Probe with appropriate primary antibodies.
10. Use Protein A-HRP for Western blot detection.
11. Incubate the membrane with Chemiluminescent-HRP substrate according to the manufacturer's instructions.
12. Expose the membrane to autoradiography film or another detection system for the appropriate time period that yields best results. For best results, expose for 30-300 sec.

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