



Title: *General Immunoprecipitation (IP) Protocol*

No: RTLP-GL-Ab20

Location:
Old CCRC Tripp Lab

Approval Date:
21 October 2005

Supersedes Date:

Materials:

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•Lab coat	•Aprotinin	•SDS-PAGE (gels and power source)	•Pipettes
•Gloves	•Leupeptin	•Microfuge tubes	•Pipetteman
•Tris	•Pepstatin	•Micro-centrifuge	•Pipette Aid
•EDTA	•Na ₃ VO ₄	•Lysates to be tested	•Pipetteman tips
•NaCl	•NaF	•Rocker or Orbital Shaker	•Phosphate Buffered Saline (PBS)
•NP-40 or Triton X-100	•Protein A or G		•Bovine Serum Albumen (BSA)
•Centrifuge	Agarose/Sepharose		
•Sodium Deoxycholate			

Prepare:

RIPA buffer: 50mM Tris (pH 8.0), 1mM EDTA (pH 8.0), 150 mM NaCl, 0.1% NP-40, 0.5% Sodium Deoxycholate, 1µg/mL Aprotinin, 1µg/mL Leupeptin, 1µg/mL Pepstatin, 1mM Na₃VO₄, 1mM NaF .

NOTE: You may substitute Triton X-100 for NP-40.

Procedure:

1. Rehydrate protein A or G Agarose/Sepharose: wash beads three times in PBS, micro-centrifuge at 14,000 rcf between washes, and resuspend in 5% BSA/PBS to 50% (v/v) slurry. Block the rehydrated beads in the BSA/PBS from 2 hours to overnight at 4°C.
2. Dilute cell lysate to 2mg/mL with RIPA buffer. Pre-clear an aliquot of cell lysate supernatant, preferably at 1 mg/mL, using 60µL rehydrated beads per 1 mL of cell lysate. Incubate mixture 1 hr at 4°C on a rocker or orbital shaker. Cell lysate supernatants of greater than 1mg/mL may be required for antigens of low expression. NOTE: An irrelevant antibody or sera of the same host and subclass as

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the IP antibody may be included in the reaction to further reduce non-specific binding.

3. Remove beads from pre-cleared lysate by centrifuging at 14,000 rcf for ~ 30 sec (quick spin). Save 20 μ L of pre-cleared cell lysate for use as a control. Transfer the remaining pre-cleared lysate (supernatant) to a fresh centrifuge tube. Used beads may be treated with sample buffer and loaded on SDS-PAGE gels as an appropriate negative control.
4. Add the suggested amount of antibody to 400 μ L of pre-cleared lysate, RIPA buffer. Incubate reaction mixture overnight at 4°C on a rocker or orbital shaker.
5. To the reaction mixture add 60 μ L of fresh Agarose/Sepharose beads (50% slurry) and incubate tubes at 4°C for 2 hours on a rocker or orbital shaker.
6. Collect IP complexes by micro-centrifuging mixture 30 seconds at 14,000 rcf, and discard the supernatant. Wash the IP complexes 5 times in RIPA buffer, microcentrifuging at 14,000 rcf between washes.
7. Resuspend IP complexes in 50 μ L 2x concentrated sample buffer and boil for 5 minutes. Centrifuge to pellet Agarose/Sepharose beads, 20 μ L per lane supernatant is loaded onto SDS-PAGE.
8. It is recommended that the original cell lysate be loaded as a positive control, and that the beads alone, pre-cleared beads, and reaction mixture (minus antibody) be loaded as negative controls.