

Immunoprecipitation Protocol

1. Dilute lysate into IP buffer (either phosphate or tris-based buffer, with up to 1% NP-40). For a single IP, prepare 250ug protein in 250-500ul total volume (use the same volume for all reactions).
2. Pre-clear lysate with Protein-A/G (depending on species and type of antibody used to IP). Incubate 4°C with rocking for at least 1 hr. This step is optional, but may decrease background.
3. Spin 12,000g x 20 seconds. Save supernatant into a new tube, discard the beads (or probe by Western if your protein of interest binds to the beads).
4. Add IP antibody at a 1:50 to 1:300 dilution (varies with the antibody). Incubate 4°C with shaking for 1 hr.
5. Add Protein-A/G (25ul slurry per 250ug lysate). Incubate O/N with rocking at 4°C.
6. The next day, wash IP beads 4 times. For mild washing, use PBS or the IP buffer. STEN buffer (detailed below) is a basic IP and wash buffer. For increased stringency, also wash in STEN with 0.5M NaCl, 1% NP-40, and 0.1% SDS. The final wash should be mild to prevent salt or detergent carry-over. Incubate with 1 mL washing buffer for 20 min at 4°C with shaking. Spin down beads 12,000g x 20 sec and carefully remove supernatant from beads (remove all of last wash using a thin pipette tip).
7. Add 2x sample buffer with BME directly to beads, and heat 100°C for 5 min. Load directly onto a gel or store at -20°C.

NOTES:

- Controls: To ensure that signal is not from non-specific binding to the beads, run a sample with all components except the antibody. To ensure signal is not from antibody, run antibody and beads without lysate.

STEN Buffer Protocol

Stock Solutions

- (1) **2 M Tris HCl, pH 6.8** (Trizma Hydrochloride, 250 mL)
 $(2 \text{ mol/L})(0.25\text{L})(157.6\text{g/mol}) = 78.8\text{g}$
- pH to 6.8: Add 13 mL 10 M NaOH, adjust accordingly.
- (2) **2.5 M NaCl** (250 mL)

$$(2.5 \text{ mol/L})(0.25\text{L})(58.44\text{g/mol}) = 36.525\text{g}$$

STEN

Stock	Volume	Final
2 M Tris, pH 7.6	25 mL	50 mM
2.5 M NaCl	60 mL	150 mM
0.5 M EDTA, pH 8.0	4 mL	2 mM
100% NP-40	2 mL	0.2%
Saturated PMSF (20mM in isopropanol)	2.5 mL	50 μ M
Leupeptin (5mg/ml in H2O)	1 mL	5 μ g/ml
H2O	905.5 mL	
	1000 mL	

0.5 M STEN

Stock	Volume	Final
2 M Tris, pH 7.6	25 mL	50 mM
2.5 M NaCl	200 mL	500 mM
0.5 M EDTA, pH 8.0	4 mL	2 mM
100% NP-40	2 mL	0.2%
Saturated PMSF (20mM in isopropanol)	2.5 mL	50 μ M
Leupeptin (5mg/ml in H2O)	1 mL	5 μ g/ml
H2O	765.5 mL	
	1000 mL	

SDS STEN

Stock	Volume	Final
2 M Tris, pH 7.6	25 mL	50 mM
2.5 M NaCl	60 mL	150 mM
0.5 M EDTA, pH 8.0	4 mL	2 mM
100% NP-40	2 mL	0.2%
Saturated PMSF (20mM in isopropanol)	2.5 mL	50 μ M
Leupeptin (5mg/ml in H2O)	1 mL	5 μ g/ml
H2O	900.5 mL	
20% SDS	5 mL	0.1%
	1000 mL	