

1. Solutions and Reagents

1.1 10X Cell Lysis Buffer (Cat. #: 3500-1)

200 mM Tris-HCl (pH 7.5)
1.5M NaCl
10 mM Na₂EDTA
10 mM EGTA
10% Triton
25 mM sodium pyrophosphate
10 mM β-glycerophosphate
10 mM Na₃VO₄ (activated)
10 µg/ml leupeptin

1.2 Add Phenylmethylsulfonyl fluoride (PMSF) to a final concentration of 1mM.
(Stock solution 100mM PMSF)

NOTE: Add fresh before each use

1.3 Goat Anti-Rabbit IgG HRP-labeled secondary antibody
(Epitomics Cat. #3050-1)

2. Preparation of Non-denaturing Cell Lysate

2.1. Adherent cells

- 2.1.1 Grow cells in a T175 flask to ~90% confluency.
- 2.1.2 Wash flask twice with cold PBS.
- 2.1.3 Add 3 ml of 1x Cell lysis buffer containing 1mM PMSF to the T175 flask.
- 2.1.4 Transfer cell lysates to Eppendorf tubes and sonicate for 10–15 seconds.
- 2.1.5 Spin at 14,000 rpm, 4 °C for 10 minutes.
- 2.1.6 Aliquot the supernatant into a new microcentrifuge tubes and store at -80 °C.

2.2. Suspension cells

- 2.2.1 Harvest and count cells.
- 2.2.2 Wash cells twice with cold PBS.
- 2.2.3 Add 30 µl of 1x Cell lysis buffer containing 1mM PMSF to 1X10⁶ cells
- 2.2.4 Sonicate for 10–15 seconds.
- 2.2.5 Spin at 14,000 rpm, 4 °C for 10 minutes.
- 2.2.6 Aliquot the supernatant into a new microcentrifuge tubes and store at -80 °C.

3. Standard Immunoprecipitation Protocol

- 3.1. Mix 200–300 µl of non-denatured cell lysate with appropriate dilutions of primary antibody
- 3.2. Incubate overnight at 4 °C with gentle rocking.
- 3.3. Add 25 µl of Protein G beads (50% bead slurry) and incubate 2 hours at 4 °C with gentle rocking.
- 3.4. Microcentrifuge for 30 seconds and discard the supernatant. Wash pellet 3 times with cold PBS.
- 3.5. Resuspend the pellet with 20 µl of 2x SDS reducing sample buffer containing β-mercaptoethanol (5%, v/v) and heat the sample at 95–100 °C for 3 minutes.
- 3.6. Briefly centrifuge the sample.
- 3.7. Load 16 µl of sample on to a SDS-PAGE gel or freeze the sample for later use (reboiled for 3 minutes prior loading onto the SDS-PAGE gel).
- 3.8. Perform Western blotting analysis using HRP-conjugated anti-rabbit IgG as secondary

antibody (1:2,000, Epitomics Cat. #3050-1). (See Epitomics WB protocol for WB procedures: <http://www.epitomics.com/support/wb.html>)

4. Optional Immunoprecipitation Protocol (for target antigens with a size of ~25 or ~50 kDa)

- 4.1.** Mix 200–300 μ l of precleared cell lysate with primary antibody.
- 4.2.** Incubate overnight at 4 °C with gentle rocking.
- 4.3.** Add 25 μ l of Anti-Rabbit IgG Beads and incubate 2 hours at 4°C with gentle rocking.
- 4.4.** Microcentrifuge for 30 seconds, and remove the supernatant. Wash pellet 3 times with cold 1XPBS.
- 4.5.** Resuspend the pellet with 20 μ l 2x SDS reducing sample buffer containing 2-mercaptoethanol and heat the sample at 95–100 °C for 3 minutes.
- 4.6.** Briefly centrifuge the sample.
- 4.7.** Load 16 μ l of sample on SDS-PAGE gel or freeze the sample for later use (reboiled for 3 minutes prior to SDS-PAGE).
- 4.8.** Perform Western blotting analysis using TrueBlot HRP-conjugated anti-rabbit IgG as secondary antibody (1:1,000 eBiosciences Cat. #18-8816-33), which preferentially detects the non-reduced form of rabbit IgG over the reduced, SDS-denatured form of IgG.