Cell Lysis and Western Immunoblotting Protocol

Web: **www.swbio.com** **Order:** **order@swbio.com**

1. Solutions and Reagents

- **10X Phosphate Buffered Saline (PBS):**
  To prepare 1 L of 10X PBS buffer: 80g NaCl, 2g KCl, 14.4 g Na_2HPO_4, 2.4g KH_2PO_4, adjust pH to 7.4.
- **1X Cell Lysis Buffer with protease/phosphatase inhibitors:**
  20mM Tris-HCl (pH 7.5), 150mM NaCl, 1mM EDTA, 1mM EGTA, 1% Triton X-100, 2.5mM sodium pyrophosphate, 1mM beta-glycerophosphate, 1mM Na_3VO_4, 10mM NaF, 1mM PMSF, 1μg/ml leupeptin, and 0.3μM aprotinin, 130μM bestatin, 14μM E-64.
- **2X SDS Sample Buffer:**
  125mM Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, 100mM DTT, 0.02% Bromophenol Blue
- **Transfer Buffer:**
  25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- **10X Tris Buffered Saline (TBS):**
  To prepare 1 L of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X)
- **Blocking Buffer:**
  1X TBS, 0.1% Tween-20 with 5% w/v BSA; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g BSA and mix well. While stirring, add 0.15 ml Tween-20 (100%)
- **Wash Buffer/TBST:**
  1X TBS, 0.1% Tween-20(100%)
- **HRP-conjugate secondary antibody**
- **Chemiluminesence reagents and film for detection**

2. Cell Lysis Protocol

Westerns are performed using cell lysates from harvested cells.

a) Treat cells by specific regulator for desired time, wash cells with 1X PBS.
b) Detach adherent cells using a cell scraper or centrifuge suspended cells, and resuspend in 1X Cell Lysis Buffer with inhibitors.
c) Remove a small volume (50 µl). To perform a protein assay, determine the protein concentration for each cell lysate.
d) To the remaining volume of cell lysate, add an equal volume of 2X SDS Sample Buffer.
e) Boil each cell lysate at 100 °C for 5 min.
f) Centrifuge at 14,000 rpm in a microcentrifuge for 5 min.
g) Load equal amounts (10–20 µg) cell lysate onto SDS-PAGE gels using loading tips, along with molecular weight markers.
h) Run the gel and transfer to nitrocellulose for western immunoblotting.

3. Western Immunoblotting Protocol

Membrane Blocking

1. Block membrane by incubating 1 hour at room temperature or overnight at 4℃ with shaking in Blocking

Solution (5% BSA in TBST (50mM Tris, 100mM NaCl, 0.1% Tween-20, pH 7.4)).

Incubation with Primary Antibody

2. Dilute primary antibody at the appropriate dilution in Blocking Solution.
3. Incubate the membrane with diluted primary antibody for 1 hour at 37℃, or 2 hours at room temperature, or overnight at 4℃ with agitation.
4. Remove antibody solution. Wash the membrane 3 times for 5-10 minutes each time at room temperature in TBST with shaking.

Incubation with Secondary Antibody

5. Incubate membrane with diluted HRP-conjugate secondary antibody (according to manufacturer's instructions) in Blocking Solution for 1 hour at room temperature with shaking.
6. Repeat Step 4.
7. Wash membrane with TBST for 2-5 minutes before proceeding Chemiluminescent Reaction.

Chemiluminescent Reaction

8. Prepare and use the Chemiluminescent substrate according to the manufacturer's instructions.
9. Immediately wrap the membrane and expose to X-ray films for 10 second to 1 hour period. The exposure time may vary according to the mount of antibody and antigen.

4. Peptide Competition Protocol

Before proceeding Western Immunoblotting, add specific Blocking Peptide（Refer to ***SAB inc.*** peptide catalog#）to the diluted primary antibody in a molar ratio of 10:1 (peptide to antibody) and incubate the mixture at 4℃ for overnight or at room temperature for 2 hours.