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Protein analyses and immunoblotting

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We use this protocol and it's working

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Abstract

This protocol describes the complete workflow for Western blotting, including cell lysis, protein quantification, SDS-PAGE, protein transfer, antibody incubation, and detection . The method allows for the detection and quantification of target proteins.

Cell Lysis

1 Prepare **1X lysis buffer:**

- 50mM HEPES pH: 7.4
- 40mM NaCl
- 2mM EDTA
- 1% Triton X
- 1.5 mM NaVo4
- 300mM NaF
- 10mM Napyrophosphate
- 10mM NaBetaGlycerophosphate

Store lysis buffer at 4C.

2 Add:

• Add 1 tablet cOmplete™ Mini Protease Inhibitor Cocktail for 10 ml lysis buffer
• Add 1 tablet PhosSTOP™ Phosphatase Inhibitor for 10 ml lysis buffer

3 Partial cell use:

• Trypsinize and harvest cells into tubes on ice
• Centrifuge at 1000 x g, 4°C, 2 min
• Resuspend in 100–200 µL lysis buffer (6-well) or 300–500 µL (10 cm dish)

Use all cells:

• Place plate on ice, aspirate media, and wash with cold PBS
• Add 100–200 µL lysis buffer per well, scrape, and collect in tubes on ice

4 • Incubate on shaker at 4°C for 15–30 min
• Centrifuge at max speed, 4°C, 10 min
• Transfer supernatant and normalize protein concentration

SDS-PAGE

5 •Select gel percentage based on protein size (e.g., 8% for >100 kDa, 12% for >20 kDa)
• Prepare 1× SDS running buffer and rinse tank
• Load 5 µL ladder and up to 30 µL of each sample (5 to 30µg proteins)
• Run at 120 V for ~150 min.

Gel Transfer

6 • Prepare transfer buffer and soak PVDF membrane (0.45 µm) in ethanol, then transfer buffer.

- Assemble membrane-gel sandwich in cassette
- Place in tank filled with transfer buffer
- Transfer at 40 V for 2 hours at room temperature

Blocking and Antibody Incubation

7
- Block membranes in 5% bovine serum albumin (BSA) in TBS-T (Tris-buffered saline with Tween-20) for 30–60 min
- Incubate with primary antibody in 5% BSA overnight at 4°C.
- Wash membranes 3× with TBST
- Incubate with secondary antibody (1:3000 in 5% milk) for 1–1.5 h
- Wash 3× for a total of 30 min.

Detection

8
- Mix equal parts of ECL reagents (Pierce)
- Incubate membrane in reagent for 1–10 min
- Visualize membrane with any Chemidoc imaging system.

Buffers:

9
To make 10 L of SDS running buffer:
- 300g Tris base
- 1440g Glycine
- 100g SDS

Recipe for 10x TBS-T
- 20L of 10x TBS-T
- 1.37 M NaCl, 200mM Tris, 1% Tween, pH 7.6
- 1,601.26 g NaCl
- 501.2 g Tris HCl
- 200 gTris Base
- 200 mL Tween

Transfer buffer:
- 15 NaOH pellets (americanbio #AB1916-01000)
- 8.8g CAPS (Sigma #C6070-1KG)
- 400 mL ethanol
- 3600 mL deionized water

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