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General western blot protocol

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Abstract

General western blot protocol

Materials

- 4-12% Bis-Tris (Invitrogen NuPAGE-SDS) mini gels (1.0 mm x 10 well or 1.5 mm x 15 well) (Invitrogen, #NP0321BOX /#NP0323BOX)
- MOPS-SDS running buffer (Invitrogen, #NP0001)
- 4x LDS buffer (Invitrogen, #NP0007)
- 10x dithiothreitol (DTT) (Thermo Fisher Scientific, #A39255)
- Li-Cor Chameleon Duo Pre-stained Protein Ladder (Li-Cor, P/N: 928-60000)
- Tris Glycine transfer buffer 10X (KD Medical, #RGF-3391)
- HPLC Methanol (Fisher Scientific, #A452SK)
- Li-Cor Intercept (TBS) blocking buffer (Li-Cor, P/N: 927-60001)
- Tris-buffered saline 10X (Corning, #46-012-CM)
- Tween20 (Sigma, #P1379)
- Revert 700 Total Protein Stain for Western Blot Normalization (Li-COR Biosciences, #926-11010)
- Primary antibodies
- Sodium azide (Sigma, #S-8032)
- IRDye 800CW Goat anti-Mouse IgG Secondary Antibody (Li-Cor, P/N: 926-32210), IRDye 680RD Goat anti-Rabbit IgG Secondary Antibody (Li-Cor, P/N: 926-68071), IRDye 800CW Donkey anti-Chicken Secondary Antibody (Li-Cor, P/N: 926-32218)
- Immun-Blot LF PVDF Membrane (BioRad, #1620264)
- Filter papers (Whatman, #1001125)
- XCell II Blot Module (Invitrogen, #EI9051)
- 1.5 mL microcentrifuge tubes
- Transfer sponges
- Aluminum foil
- Li-COR Odyssey M

Protocol materials

REVERT Total Protein Stain Kit **LI-COR Catalog #**926-11010

Li-Cor Intercept (TBS) blocking buffer (Li-Cor, P/N: 927-60001) **LI-COR Catalog #**P/N: 927-60001

Sample preparation

1 Prepare appropriate cellular extract for analysis (for example, see **Transfecting using FuGENE® 4K Transfection Reagent and cell lysis (M-PER) in a 6-well plate** protocol).

2 Measure the protein content of each sample (for example, see **Bradford Assay** protocol).

Run gel

3 Add all to a 1.5 mL microcentrifuge tube:

For loading a 1.0 mm x **10 well**, 4-12% Bis-Tris (Invitrogen NuPAGE-SDS) mini gel (Invitrogen, #NP0321BOX):

- 5 µL 4x LDS buffer (final concentration : 1X)
- 2 µL DTT (final dilution is 1:10)
- Amount of total protein for each sample (calculate from Bradford Assay, 5-20 µg is typical)
- add H2O to 20 µL (final volume)

For loading a 1.5 mm x **15 well**, 4-12% Bis-Tris (Invitrogen NuPAGE-SDS) mini gel (Invitrogen, #NP0323BOX):

- 2.5 µL 4x LDS buffer (final concentration : 1X)
- 1 µL DTT (final dilution is 1:10)
- Amount of total protein for each sample (calculate from Bradford Assay, 5-20 µg is typical)
- add H2O to 10 µL (final volume)

4 Briefly spin the microcentrifuge tubes using a tabletop micro-centrifuge to collect the sample at the bottom of the tube and then load onto the gel. Add 5-10 µl of Li-Cor Chameleon Duo Pre-stained Protein Ladder to Lane 1 for relative molecular weight markers and sample orientation.

Boil samples for 5 min. at 100°C if needed.

5 Run gel at 100 volts in 1X MOPS-SDS running buffer for 00:50:00 50m

6 Prepare transfer buffer solution at room temperature:

To make 2 L:
- 200 mL 10X Tris-glycine transblot buffer
- 400 mL 100% methanol
- ddH2O to 2 L

7 Pre-wet PVDF membrane for 00:00:30 with 100% methanol, then soak in transfer buffer for about 1 hour. 30s

8 Soak transfer sponges and Whatman filter paper in transfer buffer for about 1 hour.

Transfer

9 Assemble the transfer apparatus:

From bottom to top:
- sponge
- Whatman paper
- gel
- PVDF membrane
- Whatman paper
- sponge

10 Transfer proteins to the membrane at 40 volts for 02:00:00 2h

Total Protein Stain

11 After transferring, completely try the membrane by placing it on a piece of Whatman filter paper and incubating in 37 °C for 00:10:00 10m

12 Stain for total protein by following the protocol for the REVERT Total Protein Stain Kit **LI-COR Catalog** #926-11010

13 Block the membranes for 1 hr using Li-Cor Intercept (TBS) blocking buffer 1h

Li-Cor Intercept (TBS) blocking buffer (Li-Cor, P/N: 927-60001) **LI-COR Catalog #**P/N: 927-60001

(enough to cover the membrane). 01:00:00

Primary antibodies

14 Decant the blocking buffer and add the appropriate primary antibody dilution.

15 Incubate the membrane and the primary antibody dilution at 4°C overnight with gentle shaking.

Secondary antibodies 2h

16 Decant the primary antibodies. Wash the membrane with 1X TBS + 0.5% Tween20 + 0.3% sodium azide for 15 minutes with gentle shaking. Repeat the wash 2 more times. 45m

00:45:00

17 Rinse the membrane briefly with ddH2O. Add secondary antibodies (1:10000) diluted in Licor blocking buffer solution + Tween20 (0.5%) and incubate for 1 hour covered in aluminum foil at room temperature with gentle agitation. 01:00:00 1h

18 After the secondary antibody incubation, wash as outlined in Step 16. Add a fourth and final wash with ddH2O for 15 minutes. 01:00:00 1h

19 Image the membrane using the Li-COR Odyssey M system.