May 23, 2022

Western blot (NuPAGE and MES buffer)

Laura Smith[1]

[1]Department of Clinical and Movement Neurosciences, Queen Square Institute of Neurology, University College London (UCL)

dx.doi.org/10.17504/protocols.io.261genwyyg47/v1

Laura Smith

Cells were lysed in 1% Triton X 100 lysis buffer with protease and phosphatase inhibitors. Cell lysates were electrophoresed with NuPage™ Bis-Tris protein gels. Proteins were transferred to a PVDF membrane, blocked in 10% milk and treated with primary and secondary antibodies in 5% milk. Antibody binding was detected using the GE Healthcare Amersham™ electro-chemi-luminescence (ECL)™ Prime Western Blotting Detection Reagent.

DOI

dx.doi.org/10.17504/protocols.io.261genwyyg47/v1

Laura Smith 2022. Western blot (NuPAGE and MES buffer). **protocols.io** https://dx.doi.org/10.17504/protocols.io.261genwyyg47/v1

protocol ,

Apr 28, 2022

May 23, 2022

61606

Gel Electrophoresis

1 Make loading dye using NuPAGE Sample Buffer (4X) and Reducing agent (10X). For 18 µL of sample, add 6 µL sample buffer and 1 µL reducing agent.

2 Combine sample and loading dye and heat at 70 °C for 10 minutes .

3 Prepare precast gel (NuPAGE) by removing comb and the tape at the bottom of the gel chamber.

4 Set up the gel chamber in the gel tank so that the well openings of the precast are facing inwards toward each other.

5 Fill the gel chamber with 250 mL((1X) NuPAGE MES running buffer.

6 Remove samples from heat and spin at 10K for 1 minute to collect any evaporated sample in bottom of tube.

7 Load samples into gel using hamilton pipette tips. Place protein ladder marker in the first or last well.

8 Run gel at 200V for 45 minutes or until the protein has migrated far enough into the gel.

Transfer

9 Prepare Towin transfer buffer: 25 mM Tris, 192 mM glycine, pH 8.3, with 20% methanol (vol/vol).
To prepare 1 L of buffer:
- 800 mL distilled H2O
- 200 mL methanol
- 3.03 g Tris base
- 14.4 g Glycine

10 Soak PVDF membrane in methanol.

11 Soak transfer sandwich components (2 sheets of filter paper and 2 pads) in Towin transfer buffer until completely damp. Use hands or roller to remove any air bubbles.

Citation: Laura Smith Western blot (NuPAGE and MES buffer) https://dx.doi.org/10.17504/protocols.io.261genwyyg47/v1

12 Make up transfer sandwich in casette being careful to avoid trapping any air between layers. Layers should be 2 pads, 1 filter paper, gel, PVDF membrane, 1 filter paper and 2 pads.

13 Place cassette in transfer tank with transfer buffer and run for 1 hour at 30V.

Blocking and immunoblotting

14 Remove membrane from sandwich, noting the orientation relative to the gel to prepare for blocking.

14.1 For analysis of alpha-synuclein, fix the membrane with 0.4% PFA and 0.01% (v/v) glutaraldehyde for 30 minutes prior to blocking.

15 Block membrane in 10% semi-skimmed milk in PBS at room temperature for 1 hour.

16 Add desired primary antibody in 5% semi-skimmed milk in PBS and incubate with membrane for 1 hour at room temperature or 4°C over-night.

17 Wash membrane twice in 0.4% PBS-Tween.

18 Add appropriate HRP-linked secondary antibodies to 5% semi-skimmed milk (1:250) and incubate with membrane for. hour at room temperature.

19 Wash membrane three times in 0.4% PBS-Tween. Wash once in PBS.

20 Incubate membrane with 2 mL GE Healthcare Amersham™ electro-chemi-luminescence (ECL)™ Prime Western Blotting Detection Reagent for 5 minutes and drain excess reagent.

Citation: Laura Smith Western blot (NuPAGE and MES buffer) https://dx.doi.org/10.17504/protocols.io.261genwyyg47/v1

21 Develop blots using the Bio-Rad ChemiDoc imaging system (Bio-Rad) using the ‘chemi’ protocol with signal accumulation mode, calibrated to the signal intensities.

Citation: Laura Smith Western blot (NuPAGE and MES buffer) https://dx.doi.org/10.17504/protocols.io.261genwyyg47/v1