Mar 19, 2025

Gel Electrophoresis and Immunoblotting

DOI
https://dx.doi.org/10.17504/protocols.io.n92ldro4ng5b/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.n92ldro4ng5b/v1

Protocol Citation: Patricia Yuste-Checa, Andreas Bracher, F Ulrich Hartl 2025. Gel Electrophoresis and Immunoblotting. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldro4ng5b/v1

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Protocol status: Working
We use this protocol and it's working

Created: March 11, 2025

Last Modified: March 19, 2025

Protocol Integer ID: 124383

Keywords: ASAPCRN, polyacrylamide gel electrophoresi, gel electrophoresi, proteins by sodium dodecyl sulfate, protein, sodium dodecyl sulfate

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000282

Abstract

This protocol details how to separate proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Native PAGE, and subsequent detection by immunoblotting.

Materials

Buffers, reagents and consumables:

- NuPAGE 4–12% Bis-Tris SDS gels (Thermo Fisher Scientific)
- NuPAGE MES SDS running buffer (20X) (Thermo Fisher Scientific)
- NuPAGE™ LDS Sample Buffer (4X) (Thermo Fisher Scientific)
- Pre-stained molecular weight SDS-PAGE marker (PageRuler Prestained Protein Ladder, 10 to 180 kDa, ThermoFisher)
- NativePAGE 3%–12% Bis-Tris SDS gel **Thermo Fisher Scientific Catalog #**BN1001BOX
- NativePAGE™ Running Buffer (20X) **Thermo Fisher Catalog #**BN2001
- NativePAGE™ Sample Buffer (4X) **Thermo Fisher Catalog #**BN2003
- NativeMark™ Unstained Protein Standard **Thermo Fisher Catalog #**LC0725
- Select Western blot transfer stacks nitrocellulose (Invitrogen)
- Tris-buffered saline (TBS, [M] 10 millimolar (mM) Tris-HCl pH 7.5 , [M] 150 millimolar (mM) NaCl) containing 0.05% Tween 20 (0.05% TBS-T)
- Power Blotter 1-Step™ Transfer Buffer (5X) **Thermo Fisher Catalog #**PB7100
- PBS-T (1x PBS with 0.1% Tween 20)
- 3% bovine serum albumin (BSA) (Merck) in TBS-T. Store at 4 °C .
- Power Blotter Select Transfer Stacks, nitrocellulose, mini **Thermo Fisher Catalog #**PB3210
- Power Blotter 1-Step™ Transfer Buffer (5X) **Thermo Fisher Catalog #**PB7100

Instruments

- Microcentrifuge for 1.5 ml reaction tubes (Eppendorf)
- Semi-dry blotting apparatus (Power Blotter XL, Invitrogen)
- Luminescence gel documentation system (ImageQuant LAS 4000 mini, General Electric)

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

1 Prepare the samples by mixing with NuPAGE™ LDS Sample Buffer (4X) supplemented with [M] 100 millimolar (mM) DTT final concentration.

2 Boil the samples for 00:05:00 . 5m

3 Brief centrifugation to make sure nothing remains in the lid due to condensation.

4 Load the samples into the pockets of a NuPAGE 4–12% Bis-Tris SDS gel, or similar and run using NuPAGE MES SDS running buffer at 140 V.

Native-PAGE

5 Prepare the samples by mixing with NativePAGE Sample Buffer (4X).

6 Load the samples into a NativePAGE 3–12% Bis-Tris SDS gel or similar and run using NativePAGE running buffer at 140 V.

Protein Transfer to a Membrane

7 Remove carefully the gel from the case. Native-PAGE gel are especially sticky and susceptible to rip.

8 Wash the gel briefly with de-ionized water.

9 Perform a semi-dry transfer by placing the gel on top of the nitrocellulose membrane provided in Power Blotter Select Transfer Stacks, nitrocellulose, mini. Wet the stack with some 1x transfer buffer (Power Blotter 1-Step Transfer Buffer 5X) and make sure to remove all bubbles using a roller.

10 Place on top the filter paper provided with the stack and remove bubbles using a roller.

11 Place the stack including your gel into an Invitrogen Power Blotter semi-dry transfer system.

12 Set the corresponding program depending on the number of gels to be transferred and the size of the proteins of interest.

13 Once the transfer is done, wash the membrane with Tris Buffer Saline, 0.05% Tween (TBS-T).

14 Block the membrane during at least 01:00:00 with blocking buffer: TBS-T 5% milk or TBS-T 3% bovine serum albumin. 1h

15 After blocking, incubate the membrane with the primary antibody in blocking buffer. Depending on the antibody, incubate from 01:00:00 - 02:00:00 at Room temperature to Overnight at 4 °C . 2h

16 Wash the membrane for 00:10:00 with TBS-T. Repeat washing three times. 10m

16.1 Wash the membrane for 00:10:00 with TBS-T (1/3). 10m

16.2 Wash the membrane for 00:10:00 with TBS-T (2/3). 10m

16.3 Wash the membrane for 00:10:00 with TBS-T (3/3). 10m

17 Incubate with corresponding horseradish peroxidase (HRP) conjugated secondary antibody in blocking buffer during 02:00:00 at Room temperature . 2h

18 Wash the membrane for 00:10:00 with TBS-T. Repeat washing three times. 10m

18.1 Wash the membrane for 00:10:00 with TBS-T (1/3). 10m

18.2 Wash the membrane for 00:10:00 with TBS-T (2/3). 10m

18.3 Wash the membrane for 00:10:00 with TBS-T (3/3). 10m

19 Place the membrane in the developer tray of an imaging system like the Amersham ImageQuant 800.

20 Develop the membrane by addition of enhanced chemiluminescence (ECL) Western blot substrate like Immobilon Forte Western HRP substrate (Merck).

21 Remove the excess of substrate and record image.