

NeoClone Gel Electrophoresis Protocol

Objective: To separate protein samples using gel electrophoresis.

Materials needed:

Sample (“Sample”)

NuPAGE 4-12% Bis-Tris MES prepoured gradient gel (“Gel”) [Invitrogen NP0322box or NPO326box]

GelCode Stain Solution (“Stain Solution”) [Pierce 24590]

1.5 ml microcentrifuge tubes (“Microcentrifuge Tubes”) [Fisher 1415-2508]

Corning Gel-Loading Tips (“Gel-Loading Tips”) [USA Scientific 1415-2508]

Gridded TC Dish (“Petri Dish”) [Fisher 08-772-6]

Yellow Tips [Fisher 02-681-444]

14 & 24cm Cellophane Sheets (“Cellophane Sheets”) [ISC BioExpress E-3077-14, E-3077-24]

Solutions:

Acetic Acid, Glacial. [Sigma A06283]

Methanol [Sigma M1775]

Multimark multicolored MW standard (“MW Standard Markers”) [Invitrogen LC5725]

Deionized H₂O (“dH₂O”)

NuPAGE® LDS sample buffer (4X), (“Sample Buffer”) [Invitrogen, NP0007] stored at 4°C

NuPAGE® MES SDS running buffer (20X), (“Running Buffer”) [Invitrogen NP0002] stored at 4°C

NuPAGE® antioxidant (“Antioxidant”) [Invitrogen NP0005] stored at 4°C

NuPAGE® reducing agent 10X (“Reducing Agent”) [Invitrogen NP0004] stored at 4°C

Glycerin (“Glycerol”) [Sigma G2289]

Equipment:

Xcell SureLock Mini-Cell system (“Mini-Cell System”) [Invitrogen EI0001, EI0020, EI0002]

BioRad Power Pac 300 (“Power Pack”) [BioRad Laboratories]

Dry bath incubator (“Heat Block”) [Fisher Scientific 11-718]

Lab-Line shaker (“Shaker”) (Fisher, Model # 1314)

10µL and 200µL [Pipetmen [Gilson Pipetman P: Rainin Instrument]

Microcentrifuge [Eppendorf Centrifuge 5415 D]

14 & 24cm Gel Drying Frames; (“Gel Drying Rig”) [ISC BioExpress E-3075-14, E-3075-24]

Binder Clips [Office supply store]

Work safety level: BSL-1, unless sample requires BSL-2 conditions; under BSL-2 conditions, all work is to be done in accordance with NIH/CDC BSL-2 laboratory practices and conditions. (**Biosafety in Microbiological and Biomedical Laboratories** 4th Edition, Publication #017-040-00547-4 US Department of Health and Human Services, pages 19-26)

Prepare the Gel:

1. Turn the Heat Block to 70°C.
2. Make 1X MES Running Buffer from 20X MES stock. 200ml is required for the upper buffer chamber, another 500ml is required for the lower buffer chamber to cool the gel.
3. Remove the comb from prepoured Gel. Remove tape from the lower lip of the Gel. Rinse Gel with dH₂O.
4. Place Gel into Mini-Cell System as per manufacturer's (Invitrogen) instructions.
5. Add 200ml Running Buffer to the upper buffer chamber and check for leaks. Then add 500ml Running Buffer to the lower chamber. Make sure both ends of the Gel are covered with Running Buffer. Just prior to starting your run, add 0.5ml Antioxidant to the upper buffer chamber, and mix. Use a syringe to rinse the Gel wells with the Running Buffer.

Prepare and Load the Sample:

1. If you have some information about the Sample, you may want to modify the above - higher percentage Gels will give you better definition for smaller proteins.
2. Use 3:1 (Sample: 4X SDS Sample Buffer). If there are multiple proteins in the Sample or a lot of junk, you may need 1:1 4X SDS to properly denature the Sample. For a stained Gel use 0.5-5µg protein per lane and for a Western blot use 0.25-2µg protein per Gel. 4X Sample Buffer and Sample are needed for each well. Ideally, you need to get an idea for the protein concentration of the Sample by doing the NeoClone Modified Bradford Protein Concentration Protocol on the Sample. If necessary, dilute the Sample with dH₂O to obtain the ideal concentration for the Gel.

Write down the following information:

<i>Lanes Used</i>	<i>Contents of Lane</i>	<i>Sample Concentration</i>	<i>Amount & Type of Buffer</i>	<i>Amount of Loaded Sample</i>
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3. Prepare the Sample(s) in Microcentrifuge Tubes. Ideal volumes are up to 15µl for a multiwell Gel; 100µl for a 1 X 2D Gel. Each Sample should contain the following: the appropriate amount of Sample, 25% 4X Sample Buffer, dH₂O to make the appropriate volume as needed, and 10% Reducing Agent (added just prior to heating). Heat for 10 minutes at 70°C on the Heat Block. Pulse spin the Sample(s) in the Microcentrifuge (any condensation should be spun down into the Sample). Place Sample(s) on ice until loading.
4. Add MW Standard Markers to your Gel: 3µl for Western transfer, 10µl for stained Gels. Add the prepared Sample to the wells of the Gel with a Gel-Loading Tip. The maximum volume of the well depends on the Gel used (check the Invitrogen guidelines). **MW Standard Markers are usually run in lane 1.**

Run the Gel

1. Connect the leads to the Power Supply. The red lead goes to anode (+) on the bottom buffer chamber and the black lead goes to the cathode (-) on the upper buffer chamber. Run these Gels at a **constant voltage** (not variable). Turn the Power Supply on.

2. Run at **200V for 35minutes** or until the **MW Standard Markers** and the Sample fronts have reached the bottom of the running Gel.
3. Turn the Power Supply off.
4. Mark the Gel unless a prestained marker was used (e.g. notch upper-right corner).

Stain the Gel (**Skip this step if you are using the Gel for a Western blot.**)

1. Place the Gel in a Petri Dish and stain according to Pierce (manufacturer's) GelCode Blue Stain Reagent protocol. Summarized: wash Gel 3 X 15 minutes with dH₂O on the Shaker at speed 3 (all Shaker steps should be at this speed). Stain for one hour with the GelCode Blue Reagent. Destain with dH₂O for 1–2 hours. For faster staining of MES Gels, Gels can be prefixed with a 50% Methanol and 7% Acetic Acid mixture for 15 minutes and then washed with dH₂O prior to staining. (For 100ml: 50ml Methanol, 7ml Acetic Acid, 43ml dH₂O)

Dry the Gel

1. Transfer Gel to 5% Glycerol solution, and allow it to soak 1-2 days. Wet two Cellophane Sheets, place one Sheet on the Gel Rig (so you have support for Gel).