HiPer® Native PAGE Teaching Kit

Product Code: HTP003

Number of experiments that can be performed: 5

Duration of Experiment: ~ 2 days

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store Native Gel Loading Dye and Prestained Protein Ladder at -20°C
- Store 30% Acrylamide-Bisacrylamide Solution, TEMED and Protein samples at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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Aim:

To learn the technique of Native PAGE.

Introduction:

“Native" or "non-denaturing" polyacrylamide gel electrophoresis (PAGE) is done in the absence of SDS. While in SDS-PAGE the electrophoretic mobility of proteins depends primarily on their molecular mass, in native PAGE the mobility depends on both the protein's charge and its hydrodynamic size. Native electrophoresis is when the protein undergoes migration without denaturation. Under native PAGE conditions proteins migrate according to their charge, size and shape. In this situation polypeptides retain their higher-order structure and often retain enzymatic activity and interaction with other polypeptides.

Principle:

Electrophoresis of macromolecules is normally carried out by applying a thin layer of a sample to a solution stabilized by a porous matrix. Under the influence of an applied voltage, different species of molecules in the sample move through the matrix at different velocities. The electric charge driving the electrophoresis is governed by the intrinsic charge on the protein at the pH of the running buffer. This charge depends upon the amino acid composition of the protein. Since the protein retains its folded conformation, its hydrodynamic size and mobility on the gel will also vary with the nature of this conformation which means that higher mobility for more compact conformations and lower for larger structures like oligomers. Polyacrylamide Gel Electrophoresis (PAGE) is the most highly resolving electrophoresis method developed for separating protein molecules based on charge-density. If native PAGE is carried out near neutral pH then it can be used to study conformation, self-association or aggregation, and the binding of other proteins or compounds. Thus, native gels can be sensitive to any process that alters either the charge or the conformation of a protein.

The Native PAGE technique consists of the following three basic steps:

1. **Preparation of acrylamide gel** - Polyacrylamide is a synthetic gel which is thermo-stable, transparent, strong and relatively chemically inert and can be prepared with a wide range of average pore sizes. It can withstand high voltage gradients and is feasible for various staining and destaining procedures and can be digested to extract separated fractions or dried for autoradiography and permanent recording. A polymer gel is formed of acrylamide monomers and the proteins are run through this gel by electrophoresis, hence this entire process is called Polyacrylamide Gel Electrophoresis (PAGE).

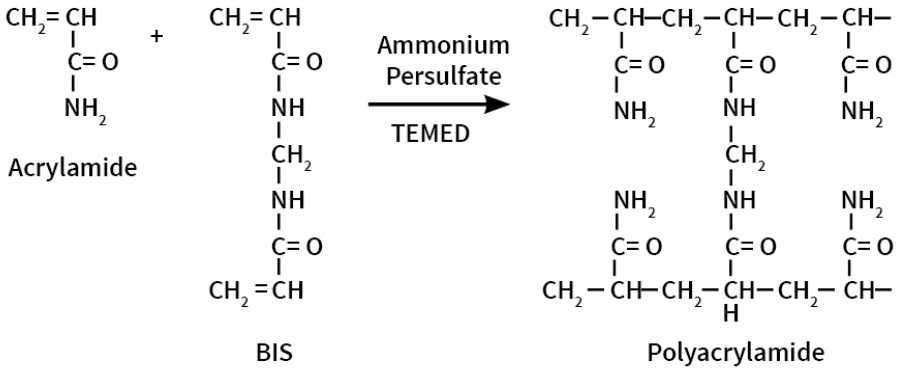


Fig1: Cross-linking of Polyacrylamide gel

2. **Electrophoresis of protein –** The porosity and the degree of crosslinking of a polyacrylamide gel depends upon the initial concentrations of Acrylamide and Bis-acrylamide which affects the resolution of the protein molecules to be separated. In a 12 – 15% Acrylamide gel large molecules are retarded during migration compared to the smaller molecules and in 4 – 8% Acrylamide gel molecules with higher molecular weight molecules have faster migration. The progress of gel electrophoresis is monitored by observing the migration of a visible tracking dye.

3. **Visualization of protein fragments –** After the completion of electrophoresis the polyacrylamide gel is stained with Coomassie® Brilliant Blue staining solution which is a methanol-based stain formulated for protein detection in polyacrylamide gel. Protein bands form an intense blue color when stained with this solution and can be easily distinguished on the gels after destaining.

Kit Contents:

The kit can be used to perform Native PAGE and visualization of migrated protein bands by Coomassie Staining.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			5 expts	
1	ML037	Acrylamide/Bisacrylamide Solution 30% (29:1)	45 ml	2-8°C
2	TKC033	1.5M Tris Solution (pH 8.8)	25 ml	RT
3	MBT092	Prestained Protein Ladder	0.030 ml	-20°C
4	TKC402	10X Tris-Glycine Gel Running Buffer	200 ml	RT
5	TKC403	Native Gel Loading Dye	0.2 ml	-20°C
6	TKC404	Protein Sample 1	0.1 ml	2-8°C
7	TKC405	Protein Sample 2	0.1 ml	2-8°C
8	DS0064	Staining solution	125 ml	R T
9	DS0065	Destaining solution	125 ml	R T
10	MB003	Ammonium persulphate (APS)	0.1 g	R T
11	MB026	Tetramethylethylenediamine (TEMED)	0.8 ml	2-8°C
12	MB002	Agarose	0.3 g	R T

Materials Required But Not Provided:

Glass wares: Conical flask, Measuring cylinder, Beaker
Reagents: Distilled water
Other requirements: Protein Electrophoresis apparatus, Micropipettes, Tips, Microwave/Burner/Hotplate

Storage:

HiPer® Native PAGE Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store the Prestained Protein Ladder and Native Gel Loading Dye at -20°C. 30% Acrylamide-Bisacrylamide Solution, TEMED and the Protein samples should be stored at 2-8°C. Other reagents can be stored at room temperature (15-25°C).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. **Preparation of 10% APS Solution:** Before starting the experiment, dissolve 0.2 g of Ammonium persulphate in distilled water to make a final volume of 2.0 ml. Store at 2-8°C. Use within 3 months.
3. **Preparation of 1X Tris-Glycine Gel Running Buffer:** To prepare 500 ml of 1X Tris-Glycine Gel Running Buffer, take 50 ml of 10X Tris-Glycine Gel Running Buffer and add 450 ml sterile distilled water*. Store at 2-8°C. Mix well before use. The 1X Tris-Glycine Gel Running Buffer can be reused 4-5 times.
4. Thaw all refrigerated samples before use.
5. Clean the entire apparatus with detergent and then with distilled water*. Ensure that the plates are free of detergent.

* Molecular biology grade water is recommended (Product code: ML064).

Procedure:

1. Assemble the electrophoresis unit such that the glass plates are clamped to the unit along with the spacers placed in-between them at two vertical edges.

2. Prepare 1% agarose (0.05g in 5ml of distilled water). Boil to dissolve the agarose and pour a thin horizontal layer at the lower edge of the plates to seal the assembly. Let it solidify by allowing it to cool down for 5-10 minutes

3. **Preparation of 12% Separating Gel-** To prepare separating gel, add the components as follows:

30% Acrylamide-bisacrylamide Solution	- 6 ml
Distilled water*	- 6.6 ml
1.5M Tris Buffer (pH 8.8)	- 2.2 ml
10% APS Solution	- 150 µl
TEMED	- 18 µl

Pour the gel in-between the plates and allow it to solidify for an hour. Immediately after the gel is poured, add distilled water to level the gel.

4. After an hour pour off the water by inverting the casting assembly.

5. **Preparation of 5% Stacking Gel-** To prepare stacking gel, add the components as follows:

30% Acrylamide-bisacrylamide Solution	- 1 ml
Distilled water*	- 4.8 ml
1.5M Tris Buffer (pH 8.8)	- 1.6 ml
10% APS Solution	- 75 µl
TEMED	- 10 µl

After addition of TEMED gently mix all the components by swirling the beaker. Pour the stacking gel on top of the separating gel and immediately place the comb avoiding air bubbles. Allow it to solidify for 30 minutes.

Note: Acrylamide is a potential neurotoxin and should be treated with great care. Always wear a face mask and use gloves.

6. Pour 1X Tris-Glycine Gel Running Buffer in the unit such that the buffer connects the two electrodes, and hence completes the flow of current. Remove the comb from the Stacking Gel carefully.

7. **Sample Preparation:** Take 2 tubes for protein samples. Label them respectively. Take 15 µl of each sample in the respective tube and add 15 µl of Native Gel Loading Dye to it.

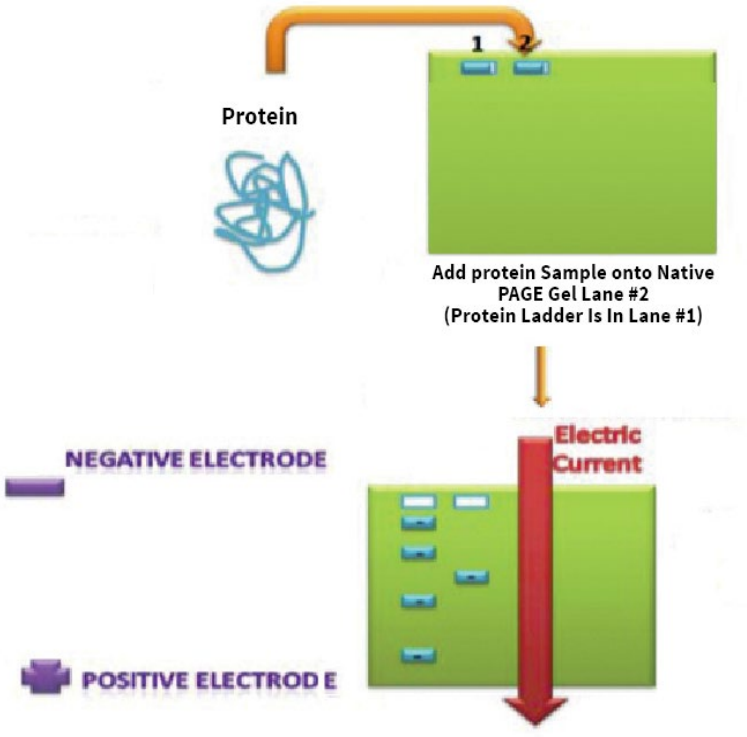
8. Load 5 µl of Prestained Protein Ladder and 20 µl of the samples in the wells created by the comb in the Stacking Gel.

9. Connect the power cord to the electrophoretic power supply according to the conventions: Red- Anode and Black- Cathode. Electrophorese at 130 volts and 90 mA until dye front reaches 0.5 cm above the sealing gel.

10. Carefully remove the gel from in-between the plates using spatula into the plastic tray containing distilled water. Wash the gel for 1 minute. Discard the water & proceed for staining destaining procedure.

* Molecular biology grade water is recommended (Product code: ML064).

Flowchart:



Proteins separate based upon charge

Staining and Destaining of Gel:

1. After removing water, add 50 ml of Staining Solution to the tray containing gel, till the bands are visible. Sometimes the gel may have to be kept overnight in the staining solution for visualization of the bands.
2. Remove the gel from Staining Solution. The Staining Solution can be re-used 2-3 times.
3. Wash the gel by rinsing with distilled water till a considerable amount of stain leaches out from the gel. Keep changing the distilled water for 3-4 times.
4. Add 50 ml of Destaining Solution to the gel. Destaining should be carried out with constant moderate shaking.
5. Continue destaining till clear, distinct bands are observed.
6. Remove the gel from Destaining Solution. Observe the gel.

Observation and Result:

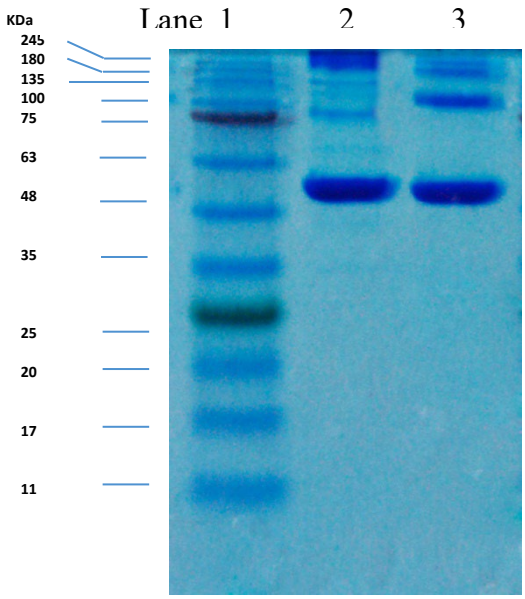


Fig 2: Gel Picture of Protein samples after Native PAGE

Lane 1: Prestained Protein Ladder
Lane 2: Protein Sample 1
Lane 3: Protein Sample 2

Interpretation:

After staining and destaining the gel compare the molecular weight of the samples with that of the protein marker. Under native conditions, separation of proteins depends on many factors including size, shape, and native charge. Since the protein retains its folded conformation, its hydrodynamic size and mobility on the gel will also vary with the nature of this conformation (higher mobility for more compact conformations, lower for larger structures like oligomers).

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Run taking unusually long time	Current is low	Increase the voltage by 25-50%
2	Poor resolution of gel	Too much sample loaded onto the wells	Never overfill the wells as it may lead to artifacts. The given volume of gel loading is for a standard gel size. If gel size smaller load samples accordingly
		Run took place very fast	Decrease the voltage by 25-50%, as current applied is very high
3	Leaking during gel casting	Chipped glass plates	Check for glass plate flaws
4	Bands on part of the slab do not move down the gel	Air bubbles between the plates underneath the affected lanes	Make sure no bubbles are present in the gel while pouring

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail at mb@himedialabs.com

Symbol:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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