HiPer® SDS-PAGE Teaching Kit

Product Code: HTP001

Number of experiments that can be performed: 10/20

Duration of Experiment: ~ 2 days

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store 5X Sample Loading Buffer and Prestained Protein Ladder at -20°C
- Store 30% Acrylamide-Bisacrylamide Solution, 2.5X Tris-SDS Buffer (pH8.8), 5X Tris-SDS Buffer (pH6.8), 5X Tris-Glycine-SDS Gel Running Buffer, TEMED and Protein samples at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

To learn the technique of SDS-PAGE.

Introduction:

SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a technique used in biochemistry, genetics and molecular biology to separate proteins according to their molecular weight. The electrophoretic mobility of proteins depends upon their size. The purpose of SDS-PAGE is to separate proteins according to their size. As proteins are amphoteric compounds, their net charge can therefore be determined by the pH of the medium in which they are suspended. Therefore, at a given pH and under non-denaturing conditions, the electrophoretic separation of proteins is determined by both size and charge of molecules. As proteins are high molecular weight molecules, it needs porous gels to get separated. Polyacrylamide gels are those which provide a means of separating proteins by size as they are porous. This kit enables the students to learn the technique of SDS-PAGE.

Principle:

To separate different protein molecules of different shapes and sizes, they first have to be denatured so that the proteins no longer have any secondary, tertiary or quaternary structure. Sodium dodecyl sulphate (SDS) is an anionic detergent which denatures proteins by "wrapping around" the polypeptide backbone. SDS denatures all the proteins to their respective primary structure. SDS confers a negative charge to the polypeptide in proportion to its length.

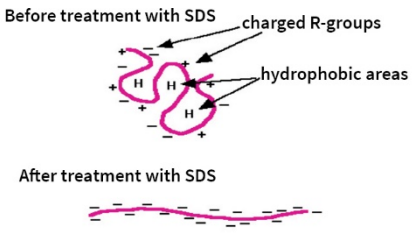


Fig 3: Denaturation of protein by SDS

Fig1: Denaturation of protein by SDS

SDS treatment has two important features:

1. All proteins retain only their primary structure.
2. All proteins have a large amount of negative charge.

Polyacrylamide is the best gel recommended to provide such an environment. 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 to 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).

There are two layers of gel, namely Stacking or spacer gel, and Separating or resolving gel.

Stacking gel - The stacking gel contains large pores of polyacrylamide gel (generally 5%). This gel is prepared with Tris buffer of pH 6.8 which is of about 2 pH units lower than that of the electrophoresis buffer. This gel is formed over the separating gel.

Separating Gel - The separating gel contains small pores of polyacrylamide gel (5-30%). The Tris buffer used is of pH 8.8. In this gel, macro molecules separate according to their size.

The materials used in SDS-PAGE and their roles are as follows:

1. **Tris:** It is used as a buffer because it is an innocuous substance to most proteins. Its pKa is 8.3 at 20°C and reasonably a very satisfactory buffer in the pH range 7.0 – 9.0.
2. **Acrylamide:** This is a white crystalline powder and while dissolving in water, autopolymerisation takes place. It is a slow spontaneous process by which acrylamide molecules join together by head on tail fashion. But in presence of free radicals generating system, acrylamide monomers are activated into a free-radical state. These activated monomers polymerise quickly and form long chain of polymers. This kind of reaction is known as Vinyladdition polymerisation.
3. **Bisacrylamide (N,N'-Methylenebisacrylamide):** Bisacrylamide is the most frequently used cross linking agent for polyacrylamide gels. Chemically it has two acrylamide molecules coupled head to head fashion at their non-reactive ends.
4. **Sodium Dodecyl Sulphate (SDS):** SDS is the most common denaturing agent used to denature native proteins to individual polypeptides. When a protein mixture is heated to 100°C in presence of SDS, the detergent wraps around the polypeptide backbone. It binds to polypeptides in a constant weight ratio of 1.4 g/g of polypeptide. In this process, the intrinsic charges of polypeptides become negligible when compared to the negative charges contributed by SDS. Thus, polypeptides after treatment become a rod like structure possessing a uniform charge density, that is same net negative charge per unit length.
5. **Ammonium Persulphate (APS):** APS is an initiator for gel formation.
6. **N, N, N', N'-tetramethylethylenediamine** (TEMED): Chemical polymerisation of acrylamide gel is used for SDS-PAGE. It can be initiated by ammonium persulfate and the quaternary amine, N,N,N',N'-tetramethylethylenediamine (TEMED).

Kit Contents:

The kit can be used to perform SDS-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
			10 expts	20 expts	
1	ML037	Acrylamide/Bisacrylamide Solution 30% (29:1)	80 ml	160 ml	2-8°C
2	ML039	2.5X Tris-SDS Buffer (pH 8.8)	65 ml	130 ml	2-8°C
3	ML040	5X Tris-SDS Buffer (pH 6.8)	20 ml	40 ml	2-8°C
4	MBT092	Prestained Protein Ladder	0.055 ml	0.110 ml	-20°C
5	ML041	5X Tris-Glycine-SDS Gel Running Buffer	400 ml	2X 400 ml	2-8°C
6	TKC037	5X Sample Loading Buffer	0.17 ml	0.34 ml	-20°C
7	TKC098	Protein Sample 1	0.24 ml	0.48 ml	2-8°C
8	TKC099	Protein Sample 2	0.24 ml	0.48 ml	2-8°C
9	DS0064	Staining solution	250 ml	500 ml	R T
10	DS0065	Destaining solution	250 ml	500 ml	R T
11	MB003	Ammonium persulphate (APS)	0.2 g	2 x 0.2 g	R T
12	MB026	Tetramethylethylenediamine (TEMED)	0.350 ml	0.7 ml	2-8°C
13	MB002	Agarose	0.6 g	1.2 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® SDS-PAGE Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store the Protein marker and 5X Sample Loading Buffer at -20ºC. 30% Acrylamide-Bisacrylamide Solution, 2.5X Tris-SDS Buffer (pH 8.8), 5X Tris-SDS Buffer (pH 6.8), 5X Tris-Glycine-SDS Gel Running Buffer and the Protein samples should be stored at 2-8ºC. Other reagents can be stored at room temperature (15-25ºC).

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 2-8ºC. Use within 3 months.
3. **Preparation of 1X Tris-Glycine-SDS Gel Running Buffer:** To prepare 500 ml of 1X Tris-Glycine-SDS Gel Running Buffer, take 100 ml of 5X Tris-Glycine-SDS Gel Running Buffer and add 400 ml sterile distilled water*. Store at 2-8ºC. Mix well before use. The 1X Tris-Glycine-SDS 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: ML024).

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*	- 3 ml
2.5X Tris-SDS Buffer (pH 8.8)	- 6 ml
10% APS Solution	- 125 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	- 1.3 ml
Distilled water*	- 5.1 ml
5X Tris-SDS Buffer (pH 6.8)	- 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-SDS 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 20 µl of each sample in the respective tube and add 5 µl of 5X Sample Loading Buffer to it. Boil the tubes containing Protein Samples at 100°C in a boiling water bath. Do not boil the tube containing Prestained Protein Ladder.

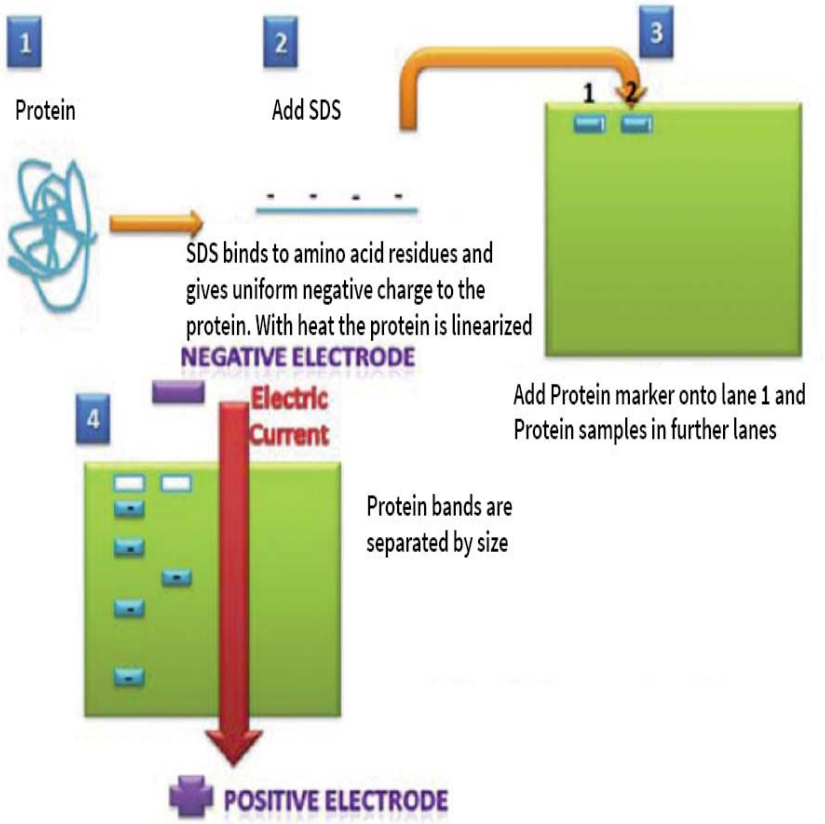
8. Load 5 µl of Prestained Protein Ladder and 20 µl of the samples immediately after the heat treatment in the wells created by the comb in the Stacking Gel.

9. Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black- Cathode. Electrophorese at 100 volts and 10 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: ML024).

Flowchart:



Staining and Destaining of Gel:

1. After removing water, add 50 ml of Staining Solution in 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 in 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. The Destaining Solution can be re-used 2-3 times.

Observation and Result:

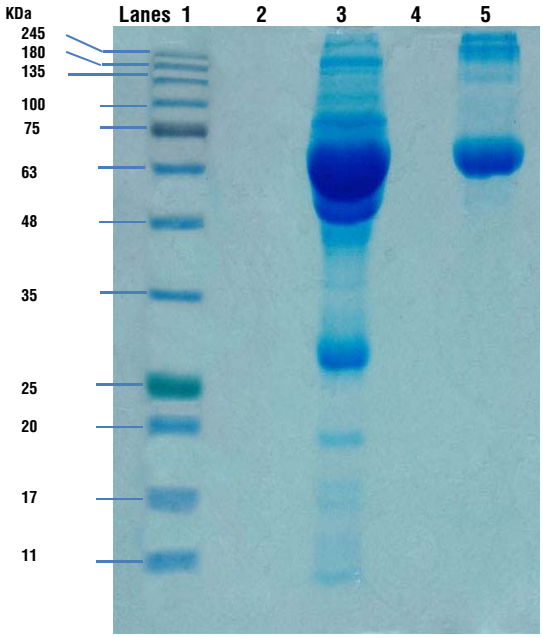


Fig 2: Gel Picture of Protein samples after SDS-PAGE

Lane 1: Prestained Protein Ladder
Lane 3: Protein Sample 1
Lane 5: Protein Sample 2

Interpretation:

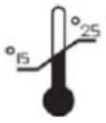
After staining and destaining the gel compare the molecular weight of the samples with that of the protein marker. Protein sample 1 contains serum hence multiple bands can be seen. Protein Sample 2 is a purified protein hence 1 major band can be seen.

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 the gel size is smaller load samples accordingly
		Length of stacking gel improper	Always pour stacking gel such that the length is 1 cm from the well bottom to the top of separating gel for proper stacking of protein sample
		Run took place very fast	Decrease the voltage by 25-50%, as current applied is very high
3	More bands seen for the purified protein sample	Proteolysis	Carry out the electrophoresis as soon as the sample is prepared
4	Leaking during gel casting	Chipped glass plates	Check for glass plate flaws
5	Samples do not sink to the bottom of the well while loading	Combs removed before stacking gel properly polymerized	Allow the stacking gel to polymerize for atleast 30 minutes before removing the combs
6	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 when pouring
7	Distorted bands observed	Excessive pressure applied to the gel plates when the gel is placed into the clamp assembly	Do not overtighten the screws on the clamp assembly
		Inconsistent pore size throughout the gel	Ensure that the gel mixture is well mixed before pouring the gel

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



Storage temperature

Do not use if package is damaged

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