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Product Name	Cat. No.	Pack Size
SDS PAGE CBB Staining Teaching KIT	**# BB-PATK010**	**10 Experiments**

AIMS:

- To learn the preparation of Sodium Dodecyl Sulphate Polyacrylamide Gel (SDS-PAGE)
- To analyze protein purity by SDS-PAGE
- To determine the molecular weight of pure protein by comparing with known protein marker

Principle:

SDS PAGE: SDS-PAGE is the most widely used method and considered as gold standard for qualitatively analysing any protein sample within a mixture of other protein samples. From SDS PAGE one can check the protein purity and also determine the molecular weight of a protein by comparing with protein standard of known molecular weight. SDS or sodium dodecyl sulphate is an anionic detergent that binds strongly to proteins with a uniform negative charge, which nullify the basic charges present on that protein.

SDS binds fairly uniformly with protein in charge to mass ratio. Generally 1.4gm of SDS binds with 1 gm of protein. As a result the protein becomes fully negatively charged and they separate according to their molecular weight within a porous acrylamide bisacrylamide matrix. There will be no differential migration based on charge.

The technique consists of three basic steps:

Step 1: Preparation of Polyacrylamide Gel:

Cross-linked polyacrylamide gels are formed by co-polymerization of acrylamide and a cross linking agent N, N'-Methylene bisacrylamide. This reaction is catalysed by TEMED, a free radical stabilizer. Free radicals promote acrylamide polymerization, and APS (Ammonium persulfate), an oxidizing agent acts as source for them. So the role of TEMED is stabilizing these free radicals in order to improve the acrylamide polymerization. Porosity of the gel matrix depends on the percentage of acrylamide, bisacrylamide used. Lower percentage of gels has larger porosity and therefore helps in larger molecule separation whereas higher percentage gels favour separation of smaller molecules.

Step 2: Electrophoresis: After the gel matrix is polymerized, it will fix into a vertical electrophoresis apparatus. Protein samples are then treated with 5X loading dye. The loading dye contains β-mercaptoethanol (which reduces the disulphide bond of the protein and makes it linear), glycerol (it increases the density and bromophenol blue which acts as tracking dye.

Step 3: Visualization of Proteins

Proteins are colorless therefore it should be stained by suitable staining dye for visualization. Coomassie Brilliant Blue R250 is most widely used staining dye.

Kit Description:

Cat. #BB-PATK010: The Kit is designed to carry out 10 SDS PAGE.

In this kit, 3 unknown protein samples along with a known protein molecular weight marker are provided. These samples are ready to use. The amount of protein is sufficient enough so that students can load each sample in triplicate in a gel.

Sufficient staining and de-staining solution are provided to stain and de-stain 10 gels. Electrophoresis equipment and transfer apparatus should be arranged by user.

Duration of experiment: Duration of the experiment is around 6-8 hours.

Kit Contents for 10 SDS-PAGE:

Sl No	Items	Quantity for 10 reactions	Storage
1.	SDS Separating Gel Mix	80 ml	4°C
2.	SDS Stacking Gel Mix	60 ml	4°C
3.	APS	0.2 gm	4°C
4.	TEMED	0.3 ml	4°C
5.	Gel Running Buffer 10X	500 ml	4°C
6.	Agarose	1.0 gm	RT
7.	Protein Molecular Weight Marker	60 µl	4°C
8.	Protein Sample 1	200µl	4°C
9.	Protein Sample 2	200µl	4°C
10.	Protein Sample 3	200µl	4°C
11.	Staining Solution	120 ml	RT
12.	De-Staining Solution	250 ml	RT

SDS PAGE CBB STAINING TEACHING KIT # BB- PATK010 10 Experiments

Materials required but not provided:

Consumables: Measuring cylinder, conical flask, Beaker, Staining Tray, Forceps

Reagents: Ultra-pure water

Instrument: Protein Electrophoresis apparatus, Micropipettes, Tips, Microwave/Burner/Hotplate, Gel rocker, Transfer (Western Blot) Apparatus.

Storage:

BioBharati SDS PAGE CBB Staining Teaching kit is stable for 6 months from the date of receipt without showing any change in performance if stored properly. Once receipt, store all the reagents at 4°C except staining and de-staining solution which should be stored at room temperature (22°C-25°C).

Important instruction:

Read the instruction carefully before starting the experiment.

- Dilute all 10 X gel running buffer provided in the kit with double distilled water (Molecular Biology grade*). Dilute 1 part of 10X buffer with 9 part of double distilled water.
- Make sure that the glass plates are detergent free (wash the plates using 70% ethanol) before pouring the gel solution within it.

  *Recommended to use Molecular Biology grade water.

Procedure:

Electrophoresis of the protein (SDS-PAGE)

1. Assemble the glass plates in electrophoresis unit as per manufacturer's instruction.

2. If your gel casting chamber requires agarose for sealing at the lower edge of the plate prepare 1% agarose (0.05g in 5ml of distilled water). Dissolve the agarose by boiling 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 10% APS Solution: Before starting the experiment, dissolve 0.1 g of Ammonium per sulphate in distilled water to make a final volume of 1.0 ml. Store at 2-8°C. Try to use within 2 months.

4. Preparation of 12.5% Separating Gel:

To prepare 5 ml of separating gel, take 5 ml SDS Separating Gel Mix, add 50µl and 5µl of TEMED. After addition of TEMED gently mix all the components by swirling the beaker.

Pour the stacking gel in-between the plates and add distilled water immediately above the gel layer to solidify the gel and allow it to solidify for an hour. After an hour pour off the water by inverting the casting assembly.

If your gel casting plate require more than 5 ml of separating solution, double the volume up to 10 ml and change the volume of APS and TEMED accordingly.

5. Preparation of 4% Stacking Gel:

Take 5 mL SDS Stacking Gel Mix; add 25µl of APS and 3µl of TEMED.

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.

!!*Both the Separating and Stacking Gel solution have acrylamide which is a potential neurotoxin and should be treated with great care. Always wear face mask and gloves while handling.*

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. Load 5 µl of known protein molecular weight marker in the first lane (lane no. 1) and same volume of unknown protein samples in rest of the lane. We are providing sufficient amount of protein so that one can load each sample in triplicate in a single gel.

8. Connect the power cord to the electrophoretic power supply unit according to the conventions: Red-Anode and Black- Cathode. Electrophorese at 100-120volts until dye front reaches 1 cm above the sealing gel.

9. Carefully remove the gel from in-between the glass plates using spatula while immersing the gel (sand-witched between the two glass plates) into the plastic tray containing de-staining solution.

10. Keep the gel for half an hour in gel rocker. After half an hour, discard the staining solution.

11. Wash the gel twice with distilled water and then pour de-staining solution to the gel and kept in gel rocker until band observed on the gel.

12. If necessary, change the destainer once.

13. After complete, de-staining, determine the molecular weight of the unknown samples with known molecular weight marker. There are 5 bands in the marker and sizes from top to bottom are

Observation and Result:

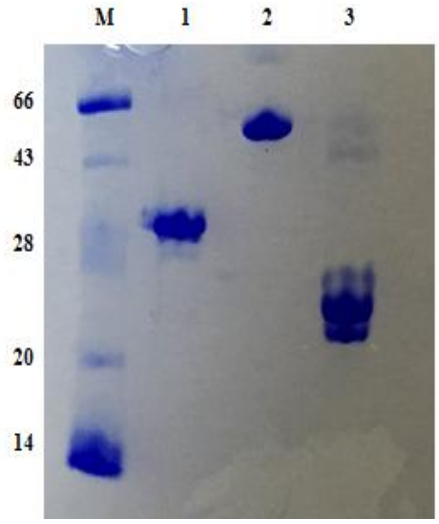

Figure 1: Analysis of protein samples in 12.5% SDS PAGE.

In the above figure, bands corresponding to 3 different protein samples were observed in SDS PAGE. Students will determine the molecular weight of the protein samples by comparing with known molecular weight marker. The above figure is a representational image which may vary with the samples provided in the kit.

Interpretation:

After complete de-staining of the gel, compare the samples with the protein ladder and determines the molecular weight of the unknown protein samples.

Trouble-shooting Guide:

Sl No	Problem	Reason for Problem	Solution
1.	Poor resolution of the gel.	Greater volume of sample loaded on the well.	Do not overload the sample in the well. Load as per the size of your well.
2.	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 at least 10-15 minutes before removing the combs
3.	Bands on part of the slab do not move down the gel	Air bubbles between the plates underneath the affected lanes	Check no bubbles are present in the gel when pouring

Technical Assistance: At BioBharati LifeScience Pvt. Ltd., we always believe to deliver the best products for quality research at our customers end. All the reagents are made at our in house R&D facility with utmost care to produce the best optimum results. We are always happy to stand beside our researcher friend at their need. For any assistance please write to us at biobharati@gmail.com or contact at our telephone no. (033)-40077640, cell 09836508080.