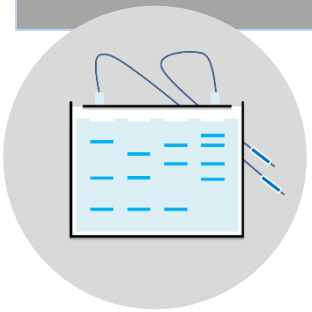


Protein Gel Electrophoresis (SDS-PAGE)

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

Gel electrophoresis is a technique in molecular biology that is used to separate and analyze macromolecules like DNA, RNA and proteins based on their size and charge. This technique makes use of the inherent mobility of charged molecules in an electric field, allowing for the separation and visualization of complex samples. Gel electrophoresis finds application in various research areas, including genetic analysis, protein characterization, and diagnostic procedures.

The principle of gel electrophoresis is grounded in the migration of charged molecules through a porous matrix or gel, driven by an applied electric field. In this method, proteins are denatured and coated with SDS (sodium dodecyl sulfate). SDS is a negatively charged detergent, which leads to a negative charge of the proteins, allowing them to be separated solely by their size during electrophoresis.

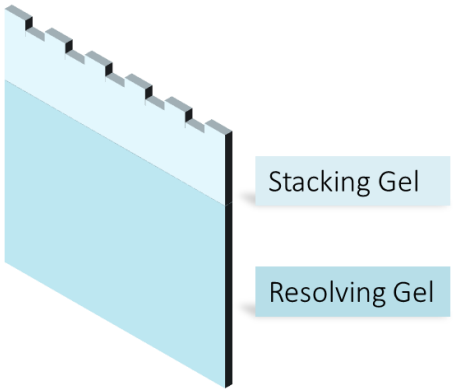
Material	
Protein sample	
Size Marker	SDS-PAGE molecular weight marker
Loading Buffer	200 mM Dithiothreitol 100 mM Tris-HCl, pH 6.8 20% Glycerol 4% Sodium dodecyl sulfate 0.25% Bromophenol blue
10 x Running Buffer	200 mM Glycine 25 mM Tris 0.1% (w/v) Sodium dodecyl sulfate Please dilute the stock solution before usage.
Protein Staining Dye	0.25 g Coomassie brilliant blue G-250 50 ml Methanol 40 ml Distilled Water 10 ml Acetic acid
Destaining Solution	50 ml Methanol 40 ml Distilled Water 10 ml Acetic acid

- 2 - Method

Gel Preparation

- Assemble the SDS-PAGE gel chamber according to the manufacturer's instructions
- Prepare the resolving gel solution as shown in the table below
- Pour the resolving gel into the gel cassette to about 2/3 of its height
- Gently pour isopropanol onto the resolving gel
- Allow the resolving gel to polymerize for at least 30 minutes
- Carefully remove the isopropanol using Filter Paper to soak remaining alcohol
- Prepare the stacking gel solution as shown in the table below
- Pour the stacking gel solution onto the resolving gel leaving some space at the top
- Insert an appropriate comb in the stacking gel
- Allow the gel to polymerize for 60 minutes
- *Note: You can create multiple gels at the same time. Please adjust the amount of reagents accordingly*

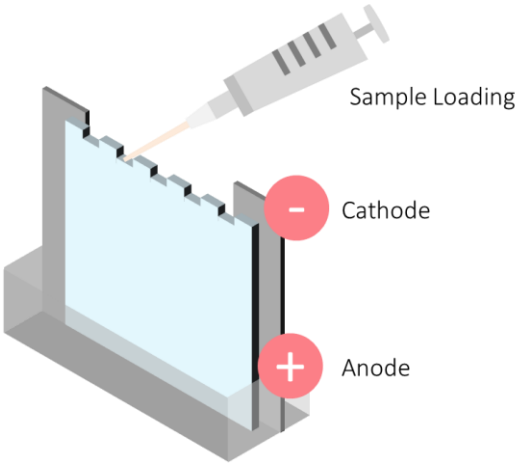
Reagent	Resolving Gel [12%]	Stacking Gel [5%]
Acrylamide solution (30%)	4 ml	1.7 ml
Distilled Water	3.2 ml	5.5 ml
1.5 M Tris-HCl, pH 8.8	2.6 ml	-
0.5 M Tris-HCl, pH 6.8	-	2.6 ml
Sodium Dodecyl Sulfate (10%)	100 µl	
	Mix the components well	
Ammonium Persulfate (10%)	100 µl	
TEMED	10 µl	
Mix gently and pour the gel solution quickly into the gel chamber. Gels can usually be stored at 2 – 8°C for several days		



Protein Sample Preparation

- Mix the protein samples with an appropriate volume of Loading Buffer in a 1:1 ratio
  i. e. 10 µl protein solution with 10 µl Loading Buffer
- Heat the samples at 95 – 100°C in a water bath for 5 minutes
- Allow the protein samples to cool down on ice

Electrophoresis



- Carefully remove the comb from the stacking gel
- Place the gel cassette in the electrophoresis chamber
- Fill the chamber with 1 x Running Buffer
- Rinse the wells with 1 x Running Buffer using a Pasteur pipette
- Load protein samples and Size Marker into the wells
- Connect the electrodes to the power supply
- Run the gel at a constant voltage of 100 V until the dye front reaches the bottom of the gel

Staining

- Turn off the power supply
- Carefully remove the gel from the cassette
- Place the SDS-PAGE gel into a staining tray
- Pour Protein Staining Dye into the container until the gel is fully submerged
- Gently shake the staining tray
- Incubate the gel over night
- Drain off the staining solution carefully
- Rinse the gel with distilled water
- Add Destaining Solution to cover the gel completely
- Gently shake the tray over night
- Rinse the gel with distilled water

Visualization

- Place the destained gel on a white background
- Capture an image of the gel using a gel documentation system or a camera
- Analyze the protein bands

- 3 - Trouble Shooting

Reason	Solution
Incorrect Gel Polymerization	Ensure thorough mixing of acrylamide with other components. Ensure that APS and TEMED are mixed well into the solution. Avoid bubbles during pouring. Use a gentle stream and let the solution flow gradually.
Protein Separation	Ensure using the correct percentage of acrylamide gel for the protein size range. 8% Gel: Suitable for resolving large proteins ranging from 100 - 2 000 kDa. 12% Gel: Suitable for resolving common proteins ranging from 10 - 250 kDa. 15% Gel: Suitable for resolving small proteins ranging from 5 – 150 kDa. 18% Gel: Suitable for resolving very small proteins usually less than 100 kDa. Adjust the running conditions, like voltage and duration, according to the gel percentage and protein size.

Reason	Solution
High Background	Use fresh Staining and Destaining Solutions. Properly destain the gel until the background clears up. Ensure the Destaining Solution is changed frequently to prevent oversaturation.
Faint Bands	Ensure proper protein loading in the wells. Check the Sample Buffer preparation to ensure proteins are fully denatured. Verify that the gel cassette was assembled correctly and there is proper contact between gel and buffer. Lower the incubation time of the destaining step.
Uneven Migration	Ensure the gel was poured evenly and with proper orientation. Verify consistent buffer conditions throughout the gel apparatus.
Artifacts	Use high-quality reagents and distilled water to prepare buffers and solutions. Prevent contamination of the gel during casting, loading and handling. Verify that the gel chamber is properly cleaned and free from debris.
Protein Degradation	Handle samples gently to avoid excessive shearing or degradation. Work at low temperatures and avoid excessive heating, which can cause protein aggregation. Consider the use of protease inhibitors.