



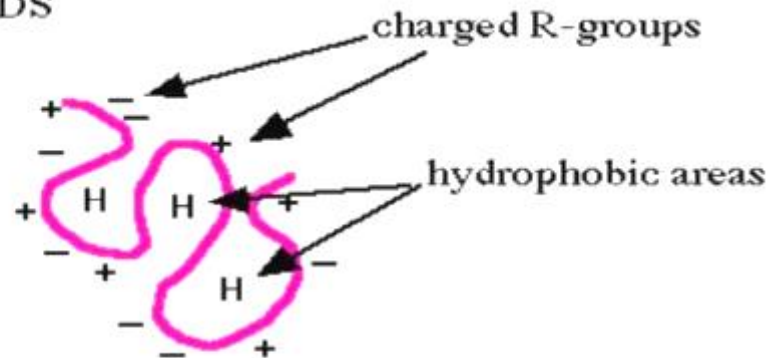
# Characterization of Proteins using SDS-Polyacrylamide gel electrophoresis

# What is SDS?



- ❖ Negatively charged detergent sodium dodecylsulfate (SDS).
- ❖ Used to denature and linearize the proteins.
- ❖ Coates the proteins with negatively charged.

BEFORE SDS



AFTER SDS





# What is PAGE?

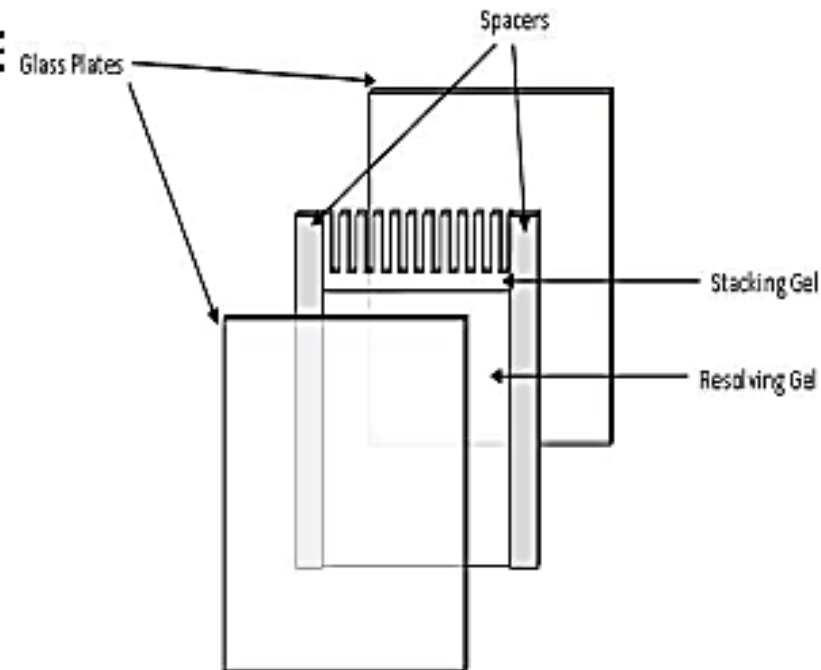
- ❖ Polyacrylamide is used to form a gel, a matrix of a pores which allow the molecules migrate at different rates.



# Preparation of gel



- Clean the plates and combs.
- Set-up the plates on the rack.
- Pour the separating gel.
- Pour the stacking gel.
- Gel storage.



SDS-Page Gel Setup

# Preparation of SDS-PAGE

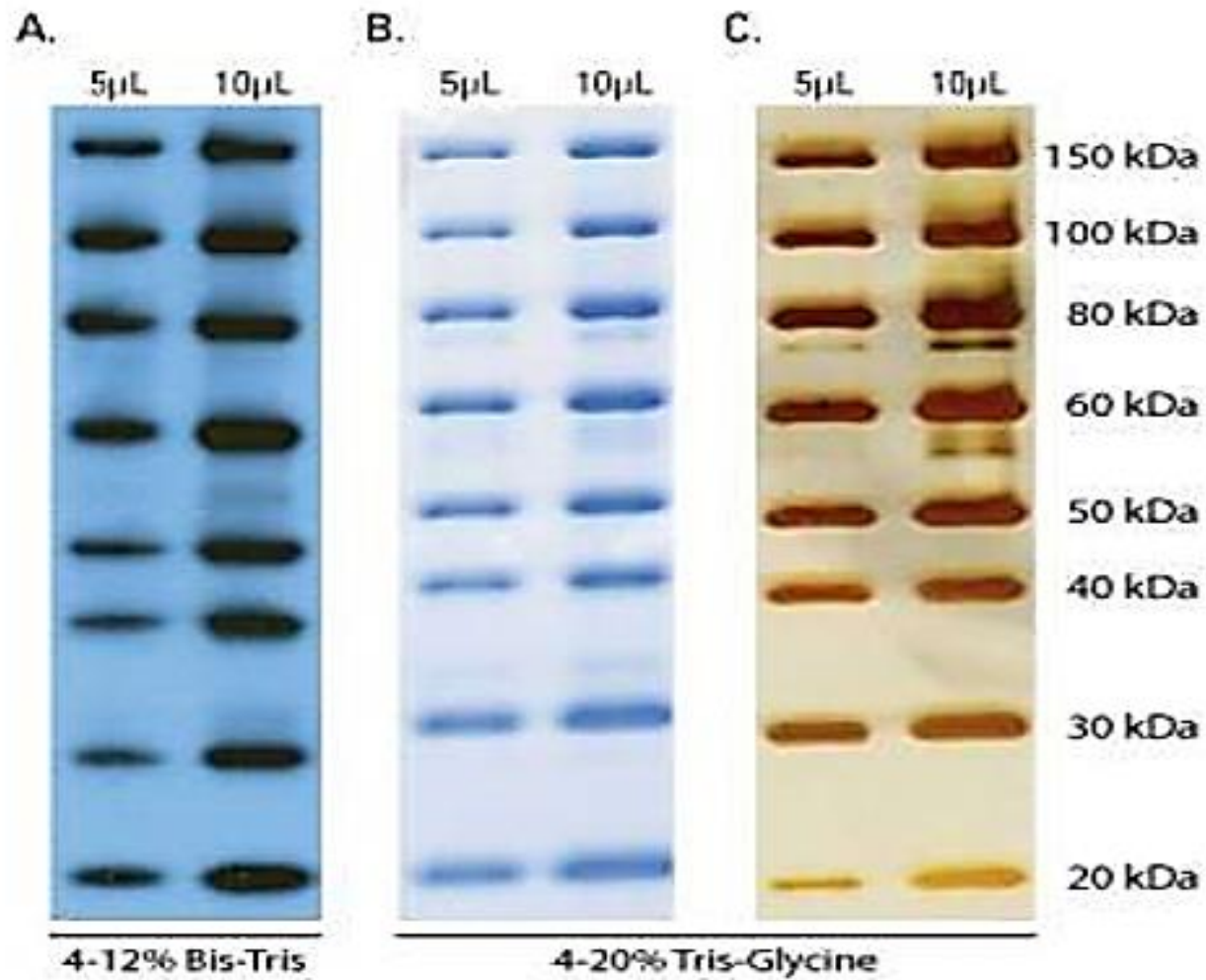


- ✓ Boil the samples for 10 minutes to completely denatures the proteins.
- ✓ Assemble the gel into the apparatus.
- ✓ Pour the buffer solution into the chamber.
- ✓ Load 20uL of samples into the well.
- ✓ After that, run electrophoresis by connecting the current supplies.

# Visualization of protein bands



- Visualizes the band under UV light
- Types of Stains:
  1. Coomassie Blue
    - Traditional method requires staining followed by destaining to remove background gel staining.
    - Most common and least sensitive
    - Limited to ~100ng of protein
  2. Silver Stain
    - Most sensitive test
    - Detection limit: 0.1-1.0ng of protein



A. Staining band with Western blot; B. Coomassie blue stain; C. Silver stain

# Chemical components of SDS-PAGE



Resolving Gel	8 %		10%	
	1 gel	2 gels	1 gel	2 gels
DDI H <sub>2</sub> O	1.8 ml	3.6 ml	1.6 ml	3.2 ml
1.5 M Tris-HCl, pH 8.8 (RG Bfr.)	1.3 ml	2.6 ml	1.3 ml	2.6 ml
40% Acrylamide Stock	800 $\mu$ l	1.6 ml	1.0 ml	2.0 ml
20 % SDS	100 $\mu$ l	200 $\mu$ l	100 $\mu$ l	200 $\mu$ l
30% Ammonium Persulfate	10 $\mu$ l	20 $\mu$ l	10 $\mu$ l	20 $\mu$ l
TEMED	4 $\mu$ l	8 $\mu$ l	4 $\mu$ l	8 $\mu$ l
	12 %		15%	
	1 gel	2 gels	1 gel	2 gels
DDI H <sub>2</sub> O	1.4 ml	2.8 ml	1.1 $\mu$ l	2.2 ml
1.5 M Tris-HCl, pH 8.8 (RG Bft.)	1.3 ml	2.6 ml	1.3 ml	2.6 ml
40% Acrylamide Stock	1.2 ml	2.4 ml	1.5 ml	3.0 ml
20 % SDS	100 $\mu$ l	200 $\mu$ l	100 $\mu$ l	200 $\mu$ l
10% Ammonium Persulfate	10 $\mu$ l	20 $\mu$ l	10 $\mu$ l	20 $\mu$ l
TEMED	4 $\mu$ l	8 $\mu$ l	4 $\mu$ l	8 $\mu$ l





## Stacking Gel (4 %)

	2 gels
DDI H <sub>2</sub> O	3.9 ml
1.0 M Tris-HCl, pH 6.8 (SG Bfr.)	500 $\mu$ l
40% Acrylamide Stock	500 $\mu$ l
20 % SDS	100 $\mu$ l
30% Ammonium Persulfate	16 $\mu$ l
TEMED	8 $\mu$ l

**Note:** Always add Ammonium Persulfate and TEMED immediately before casting gel.

Number of gels refers to 1 mm thick gels. Use the 2 gel recipe for 1.5 mm thick gels.

# Silver staining procedure



- ✓ Fix the gel in fixation solution (40% ethanol, 10% acetic acid, 50% water) for 30 minutes.
- ✓ Treat the gel with protein treatment solution (20% ethanol, 5% acetic acid, 75% water, 4 mg dithiothreitol) for 30 minutes.
- ✓ Rinse the gel with 0.5% dichromate for 5 minutes.
- ✓ Wash the gel with water for 5 minutes.
- ✓ Equilibrate the gel with 0.1% silver nitrate for 30 minutes.
- ✓ Briefly wash the gel with water for 1 minute.
- ✓ Incubate the gel in complex formation solution 0.02% paraformaldehyde, 3% sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), pH 12.
- ✓ Add 1% acetic acid to stop the complex formation.
- ✓ Visualize the stained protein bands under gel documentation system.