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SDS-PAGE

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol shows the steps carried out by team Tec-Chihuahua to perform SDS-PAGE

Materials

SOLUTIONS:

- **Acrylamide/Bis 30%**
- 29.2 g of Acrylamide.
- 0.8 g of Bis Acrylamide
- Dissolve in 50 mL of distilled water in constant agitation, gauge to a volume of 100 mL.
- Store at 4 °C degrees while protected from light.

- **Tris-HCl/ SDS 4X pH 8.8 (1.5 M Tris-HCl, 0.4% SDS)**
- 18.17 g of TRIZMA® base
- 0.4 g of SDS
- Dissolve in 80 mL of distilled water
- Adjust to a pH de 8.8 with HCl and gauge to a 100 mL
- Filtrate the solution
- Store at 4 °C degrees

- **Tris-HCl/ SDS 4X pH 6.8 (0.5 M Tris-HCl, 0.4% SDS)**
- 6.06 g de TRIZMA® base
- 0.4 g de SDS
- Dissolve en 80 mL of distilled water.
- Adjust to a pH de 6.8 with HCl and gauge to a 100 mL
- Filtrate the solution
- Store at 4 °C degrees

- **10% Ammonium Persulfate**
- 500 mg of APS (Ammonium Persulfate)
- Dissolve en 5 mL of distilled ultra pure water
- Store at -20 °C for up to two weeks
- Store at 4 °C degrees for one use only

- **2X Loading Buffer**
- 2 mL of glycerol
- 400 uL of Mercapto
- 0.02 g of bromophenol blue
- 0.4 g of SDS
- 2.5 mL of 8.6 pH buffer

- **1X Running Buffer**
- 14 g of glycine.
- 3 g of Trizma base.
- 1 g of SDS.

- Gauge to a 1 L.

- **Staining Solution**
- 1.25 g of brilliant blue R in 250 mL of methanol
- 200 mL of distilled water.
- 50 mL of Glacial Acetic Acid
- Store in room temperature while protected from light

- **Destaining Solution**
- 250 mL of methanol
- 62.5 mL of Glacial Acetic Acid
- 312.5 mL of distilled water.
- Store in room temperature

Usage: Pour solution into a container and allow to stir until the SDS gel is clear or electrophoresis bands are visible.

Troubleshooting

Protocol

3h 45m

1 Clean the components of the electrophoresis camera with 70% ethanol and gauze 's.

2 Assemble the chamber and check that there are no leaks by pouring distilled water between the glasses.

3 Prepare polyacrylamide gels:

	A	B
	Reagents	2 minigels
	Distilled water	3.4 mL
	Acrylamide/ Bis 30%	4 mL
	Tris-HCl/SDS 4X pH 8.8 (1.5 M Tris-HCl, 0.4% SDS)	2.5 mL
	ASP 10%	100 µL
	TEMED	4 µL

Separation gel (12%)

4 Pour the solution between the glasses with a 1 mL micropipette, leaving a space of 1.5 cm for the concentrating gel. To level, distilled water is added and allowed to settle for 00:30:00 or until a line is seen between the gel and the water.

30m

	A	B
	Reagents	2 minigels
	Distilled water	2.7 mL

	A	B
	Acrylamide/ Bis 30%	1 mL
	Tris-HCl/ SDS 4X pH 8.8 (1.5 M Tris-HCl, 0.4% SDS)	1.3 mL
	ASP 10%	50 µL
	TEMED	4 µL

- Concentrating gel (6%)

5 Pour solution onto separating gel using a 1 mL micropipette.

6 00:30:00 00:30:00 Insert the comb (carefully avoiding the formation of bubbles) and leave to solidify for 00:30:00 1h 30m

7 When the gels are polymerized, prepare an electrophoresis chamber with 1X running buffer until it covers the gels and it reaches the line of two gels.

8 Sample PTake the pellets contained in Eppendorf tubes
- Take the pellets contained in Eppendorf tubes
- Add 300 µL of 1X loading buffer and resuspend the pellet.reparation:

9 To denature proteins, heat samples in boiling water for 5 min.

10 Load gels with the hot sample.

11 In the first well add 7 µL of the molecular weight marker.

12 Once the samples are loaded, run the gel at 80 V for 00:20:00 and then at 180 V for 00:45:00 1h 5m

13 Turn off the camera and disarm it.

14 Remove the gels from the glasses and place in a container with staining solution. Leave stirring for one hour.

15 Remove staining solution after one hour, add destaining solution and leave stirring for 00:20:00 Change the destaining solution and leave again for 00:20:00 40m

16 Leave stirring until the gel is transparent.

17 Analyze the gels.