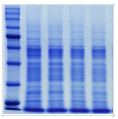

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Polyacrylamide Gel Electrophoresis (SDS-PAGE)

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1 Works for me dx.doi.org/10.17504/protocols.io.9b7h2rn

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ABSTRACT

Parameters adjusted for gels 7 cm and 1.5 mm thick.

MATERIALS

NAME	CATALOG #	VENDOR
Bromophenol Blue	BP11525	Fisher Scientific
Ammonium Persulfate, 25g (Ammonium Persulphate)	V3131	Promega
SDS	161-0302	BIO-RAD
1-Butanol	BC1800.SIZE.1L	Bio Basic Inc.
Glycine	50046	Sigma
Glycerol	104092	Merck Millipore
TEMED	View	Sigma Aldrich
HCl	View	
Tris	17926	Thermo Fisher
Coomassie Brilliant Blue G-250 Dye	20279	Thermo Fisher
500 ml 30% acrylamide and bis-acrylamide solution 29:1	#1610156	

SAFETY WARNINGS

Wear personal protective equipment: gloves, lab coat and mask.

BEFORE STARTING

Organize your workspace

Make sure all solutions and equipment are available.

Preparation of solutions and workspace

1 **Separation Gel Buffer (150 mL)**
1.5 M Tris-HCl, pH 8.8

- Tris (18,15 g/100 mL) ..... 27,23 g
- diH_2O ..... 80 mL
- Adjust pH to 8.8 with HCl
- diH_2O ..... to 150 mL
- Store at 4 ° C

2 **Stacking Gel Buffer (100 mL)**
0.5 M Tris-HCl, pH 6.8

- Tris ..... 6,00 g
- diH_2O ..... 60 mL
- Adjust pH to 6.87 with HCl
- diH_2O ..... to 100 mL
- Store at 4 ° C

3 **10% SDS (100 mL)**

• SDS ..... 10.00 g
• diH2O ..... 90 mL
• Solubilize
• diH2O ..... to 100 mL

4 **10% Ammonium Persulphate (APS) (1 mL)**

• APS ..... 0.10 g
• diH2O ..... 1 mL

Prepare at time of use

5 **10x Running Buffer (1 L)**

• Tris base ..... 30.30 g
• Glycine ..... 144.10 g
• SDS ..... 10.00 g
• diH2O ..... for 1 L
• Do not adjust pH (~ pH 8.3)

6 **Sample Buffer (8 mL)**
2X: 62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue

• 0.5 mM Tris-HCl, pH 6.8 ..... 1.0 mL
• 25% glycerol ..... 2.0 mL
• 1.0% bromophenol blue ..... 0.08 mL
• 10% SDS ..... 1.6 mL
• diH2O ..... 2.92 mL

Preparation of Denaturing Gels (SDS-PAGE)

7 Choose which concentration of separation gel to use and separate two erlenmeyer (one for separation gel and one for stacking gel)

These volumes are standardized for making two 7 cm wide and 1.5 mm thick gels.

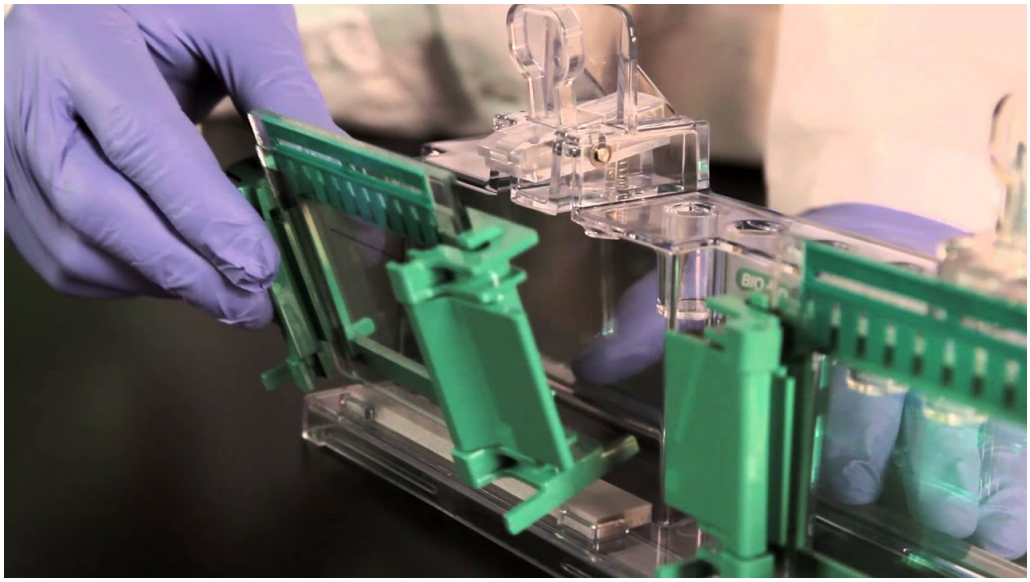
Table 1. Preparation of 20 mL SDS-PAGE Running Gel (2 x 1.5 mm gels) and 10 mL Stacking Gel (2 x 1.5 mm gels)

	Stacking Gel	Separation gel			
	4%	7,5%	10%	12%	X%
30% acrylamide/bis	1320 µL	5000 µL	6666 µL	8000 µL	0,666 x X µL
0,5 *M* Tris-HCl, pH 6,8	2520 µL	-	-	-	-
1,5 *M* Tris-HCl, pH 8,8	-	5000 µL	5000 µL	5000 µL	5000 µL
10% SDS	100 µL	200 µL	200 µL	200 µL	200 µL
diH2O	6000 µL	9700 µL	8024 µL	6700 µL	14,69-(0,666 x X) µL
10% APS	50 µL	100 µL	100 µL	100 µL	100 µL
TEMED	20 µL	20 µL	20 µL	20 µL	20 µL
Total volume	10 mL	20 mL	20 mL	20 mL	20 mL

TEMED should only be added just before spilling the solution between plates.

Gel Assembly

8



Clean the workbench, glass plates, spacers, combs and other equipment that will be used with alcohol and assemble the plates

Apply a thin layer of Vaseline to the rubber of the mounting bracket. Check for leaks by adding water. Fully remove water with filter paper

9 **Prepare the separation gel** go to step #7 **Table 1**

Add TEMED

Pour the separating gel solution onto the plate up to the demarcated limit for the stacking gel.

Apply a thin layer of n-butanol over the separation gel to level the upper portion of the gel.

Do not leave n-butanol in contact with gel for more than 1 h
Keep polymerization cassette level

Wait for complete gel polymerization, following what was left of the solution in the erlenmeyer

10 Remove n-butanol and wash with water

Dry well

11 **Prepare the stacking gel** go to step #7 **Table 1**

Add TEMED and apply the stacking gel to the separation gel (already polymerized)

Immediately put the comb in the gel being careful not to bubbles

Expect full gel polymerization

If necessary, the gel may be stored overnight in running buffer at 5 ° C.

Remove the comb only when mounting the running bowl

Sample Preparation

12 Calculate the sample volume required to provide the desired amount of protein (≈ 30 µg / channel)

Distribute the calculated sample volume into 600 µL microtube

Add 2X sample buffer to samples at a 1:1 ratio.
If sample buffer 4X, use 3:1 ratio (in this case apply for each channel 5 µL of sample buffer, add sample and complete with diH2O to 30 µL on 1.5 mm gel).

13 Boil the mix (samples plus sample buffer) for 5 min.
100 °C

Application of Samples

14 Attach the plates to the support (gel plate and dead plate).

15 Place running buffer in the space between the plates (≈ 30 mL).

Check for possible leaks.

16 Apply the samples in their respective channels.

Add sample buffer to empty channels, if any.

17 Put running buffer in the bowl halfway.

18 Fit the electrodes and turn on the device for running, using 200 V.

Expected current of gel: initial 35 - 50 mA and final 20 - 31 mA.

19 Follow the race for bromophenol blue.

Stop the race when bromophenol blue reaches the lower limit of the gel.

Do not let bromophenol blue escape completely from gel. Low-mass proteins or peptides may be lost.

20 Remove the gel from the plates.

Mark the gel to reference which side is right and which side is left.

Gel Fixation

21

Place the gel in a fixative solution (10% methanol, 5% acetic acid) and keep stirring for 2 h

Gel Development (coomassie)

22 **Coomassie Staining**

Discard the fixative solution

Add Coomassie's solution, which is:

- Ammonium sulfate (10%): 100 g
- Coomasie G250 (0.1%): 1 g
- Water: 800 mL
- Phosphoric acid (2%): 20 mL
- Methanol (20%): 200 mL

Add ammonium sulfate and Coomassie G250 to 400 mL of water until the sulfate dissolves.

Add phosphoric acid, followed by methanol slowly. Finally, add the other 400 mL of water.

Gel Development (silver)

23 **Silver staining**

Discard the fixative solution and perform three washes of 10 min with 50% ethanol (92% commercial ethanol may be used in this step)

Incubate the gel with 0,02% sodium thiosulphate solution for 1 min.

Wash the gel in distilled water 3 times

Incubate the gel in silver nitrate solution (0.2 g silver nitrate; 0.037 mL 37% formaldehyde; 100 mL water) for 20 min

Wash the gel for 20 s with water 3 times

Apply developer solution (2 g of sodium carbonate; 1 mL of 0.02% sodium thiosulfate solution; 0.025 mL of 37% formaldehyde; complete with water to 50 mL)

Wait for the bands to appear in a few seconds. In some cases, it takes a few minutes for the bands to become evident.

Once the gel reaches the required standard, stop the reaction by adding 3 mL of acetic acid.