SDS-PAGE

Solutions & Materials

Note: Stand, clamps, combs etc. were purchased by BIO-RAD (Mini-PROTEAN®)

Mini-Gels (four)		
Ingredient	Stacking gel (≈3%)	Separating gel (≈10%)
dd H_2O	6,1 ml	8 ml
0,5 M Tris-HCL pH 6.8	2,5 ml	-
1,5 M Tris-HCL pH 8.8	-	5 ml
30% Acrylamide	1,1 ml	6,8 ml
20% SDS	50 µl	100 µl
0,5 M EDTA pH 8	50 µl	-
APS (100 mg/ml)	200 µl	100 µl
TEMED	20 µl	12 µl
Final	≈ 10 ml	≈ 20 ml

5x SDS Stock		1x Running buffer	
Ingredient	Amount	Ingredient	Volume
Tris	75,1 g	5x SDS Stock	200 ml
Glycin	360,35 g	20% SDS	5 ml
dd H_2O	5000 ml	0,5 M EDTA	2 ml
		dd H_2O	793 ml
Final	5 l	**Final**	1 l

2x SDS loading buffer		
Ingredient	Volumes	Final concentration
1 M Tris pH 6.8	600 µl	60 mM
Glycerol	5 ml	50%
20% SDS	1 ml	2%
Bromphenolblue	small tip of spatula	-
1 M DTT	1 ml	100 mM
H_2O	2,4 ml	-
Final	10 ml	-

Other materials: isopropanol, 70% Ethanol, dd H_2O

Working with gloves is necessary – Unpolymerized acrylamide is toxic

Pouring gels:

1. Wash glass plates with water and 70% Ethanol.
2. Assemble glass plates into the pouring device.
3. Fill with dd H_2O to check if the construction is leaking.
4. Dry the space between the glass plates with Whatman-paper.

5. Prepare the solutions for the mini gels without adding APS & TEMED (these ingredients start the polymerization).
6. Add APS & TEMED to the **separating gel** solution (the lower gel).
7. Pour the separating gel solution in between the glass plates. Let 2,5 – 3 cm free space on the upper part of the glass plates.
8. Overlay with isopropanol using a pasteur pipette.
   Let polymerize for 45 – 60 minutes.
9. Decant the isopropanol, rinse the space between the plates with dd H_2O, dry with Whatman-paper (avoid touching the gel as it may rip apart)
10. Add APS & TEMED to the **stacking gel** solution (the upper gel)
11. Pour solution onto the separating gel, slide in the combs (avoid bubbles).
    Let polymerize for 30 – 45 min.
12. Remove comb. If necessary, mark the pockets on the outer glass.

Storage: Fill the pockets with 1x Running buffer. Wrap up the gels in wet paper tissues (dd H_2O) and store them in the refrigerator (4°C).

<u>Loading & running</u>

For electrophoresis the Mini-PROTEAN Tetra Vertical Electrophoresis Cell (BIO-RAD) was used.

1. Use the 2x SDS loading buffer for your sample (as described in the protocol you used to prepare your proteins).
2. Assemble glass plates with gels into the running device and place it into the running chamber. If you have an unequal number of gels, you need to use a buffer dam.
3. Fill the chamber with the 1x running buffer. (The pockets should also be filled with the running buffer)
4. Use 3 µl marker (PageRuler™ Prestained Protein Ladder by Thermofisher Scientific #26616) for the first pocket.
5. Load your samples into the remaining pockets.
6. Close the device. Make sure the cables are plugged in right.
7. Conduct electrophoresis at 150 V for 55 minutes or until the samples have reached the bottom of the gel.
8. Turn off the device. Disassemble your gel. Open the gel by using a spatula as a lever in between the glass plates.
9. Use the gel for further experiments.