SDS – PAGE protocol

Mini-PROTEAN Cells (BioRad)
30% Acrylamide/Bis Solution 37.5:1 (BioRad - #1610158)
Quick Coomassie stain (Serva - 35081.01)
TEMED

Preparation of chemicals:

0,5M Tris-HCl pH6,8 – 6,057g Tris base/100ml miliQ H_2O (pH adjusted with 5M HCl)

1,5M Tris-HCl pH8,8 – 18,171g Tris base/100ml miliQ H_2O (pH adjusted with 5M HCl)

10% SDS – 10g/100ml miliQ H_2O

10% APS (as peroxoaminosulphate) – 0,1g/1ml miliQ H_2O 1 month max! fridge

Isobutanol, water saturated – 20 ml + 20 ml + shake (IsobutOH in upper phase)

Preparation of solutions:

5x Running buffer
1g SDS
3g Tris base
14,4g Glycine
up to 200ml miliQ H_2O

5x Sample loading buffer
1,2 ml milliQ H_2O
0,5 ml of 0,5M Tris-HCl ph6,8
0,8 ml glycerol
0,8 ml 10% SDS
0,2 ml β-mercaptoEtOH
pinch of Bromphenol blue

Preparation of gels

- if you plan to make more gels in a short time period, you can prepare a larger amount of the solutions (without APS and TEMED) and keep them in the fridge (max 1,5 months).

Resolving gel stock (12%; 5 ml/gel)

Component	**1 gel**	**2 gels**	**4 gels**
30% A/B	2 ml	4 ml	8 ml
1.5M Tris-HCl pH 8.8	1.25 ml	2.5 ml	5 ml
10% SDS	50 µl	100 µl	200 µl
miliQ H2O	1.68 ml	3.35 ml	6.72 ml

Stacking gel (4%; 2.5 ml/gel)

Component	**1 gel**	**2 gels**	**4 gels**
30% A/B	0.33 ml	0.66 ml	1.32 ml
0.5M Tris-HCl pH 6.8	0.63 ml	1.26 ml	2.52 ml
10% SDS	25 µl	50 µl	100 µl
miliQ H2O	1.5 ml	3 ml	6 ml

Pouring the gel:

- Set up the gel tray(s)
- Pour 5ml of Resolving gel stock (per gel) into the 12% AB 15ml falcon tube
- Add 50 µl of 10% APS and 8 µl of TEMED quickly and mix well
- Immediately fill the gel tray up to 1 cm under the teeth (chambers)
- Carefully overlay the gel with 300 µl isobutanol using a syringe
- Let gel polymerize for 1 hour
- Absorb isobutanol using absorbent paper, rinse with dH_2O, and dry with absorbent paper again
- Pour 2,5ml of Stacking gel stock (per gel) into the 4% AB 15ml falcon tube
- Add 25 µl of 10% APS and 4 µl of TEMED quickly and mix well
- Immediately fill the gel tray and insert the teeth (chambers)
- Let gel polymerize for 30 min
- Remove the teeth, transfer the gel into the running apparatus, and fill with 1x Running buffer

Prepping the samples and running the gel:

- Measure protein concentration in samples. In the case of cell lysates/CFEs, each sample of 20 µl should contain 8 µg of protein; for purified proteins, 4 µg is enough.
- Mix your sample in Eppendorf tube with 5x Sample loading buffer (final conc. 1x) - usually, a sample is mixed with water to give 20 ul, and then 5 ul of loading buffer is added (12.5 ul is eventually loaded in a single well of the gel)
- Boil samples at 95°C for 5 min and centrifuge them briefly
- Load samples and 5 µl of protein marker (keep on ice!)
- Run the gel at 125 V, constant V until the loading dye (dark blue) reaches the end of the gel (ca 80 min)

Staining the gel:

- Disassemble the running apparatus, lift the small glass piece, and cut off the separating gel
- Carefully push the gel into a small container with dH_2O
- Wash 10 min
- Discard dH_2O, add around 40 ml Quick Coomassie stain, and stain for 1 hour +
- Discard the stain (into a 50ml falcon tube for reuse)
- Rinse the gel with dH_2O a few times
- Leave to de-stain in dH_2O ON