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 milliQ H_2O

1,5M Tris-HCl pH8,8 – 18,171g Tris base/100ml milliQ H_2O

..pH adjusted with 5M HCl

10% SDS – 10g/100ml milliQ H_2O

10% APS (as peroxoaminosulphate) – 0,1g/1ml milliQ 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 milliQ 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

Stacking gel stock (4%) 1,5 month! fridge
1,98 ml 30% A/B
3,78 ml of 0,5M Tris-HCl ph6,8
150 µl 10% SDS
9 ml milliQ H_2O

Resolving gel stock (12%) 1,5 month! fridge
6 ml 30% A/B
3,75 ml of 1,5M Tris-HCl ph8,8
150 µl 10% SDS
5,03 ml milliQ H_2O

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 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, 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 gel tray and insert the teeth (chambers)
- Let gel polymerize for 30 min
- Remove the teeth, transfer the gel into the running apparatus, fill with 1x Running buffer

Prepping the samples and running the gel:

- 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 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 gel at 25mA until the loading dye (dark blue) reaches the end of the gel

Staining the gel:

- Disassemble the running apparatus, lift the small glass piece and cut of 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, 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