**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 H2O

 **1,5M** **Tris-HCl pH8,8** – 18,171g Tris base/100ml milliQ H2O

 ..pH adjusted with 5M HCl

 **10% SDS** – 10g/100ml milliQ H2O

 **10% APS (as peroxoaminosulphate)** – 0,1g/1ml milliQ H2O 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 H2O

 **5x Sample loading buffer** 1,2 ml milliQ H2O

 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%)** 1,98 ml 30% A/B 1,5 month! fridge

 3,78 ml of 0,5M Tris-HCl ph6,8

 150 µl 10% SDS

 9 ml milliQ H2O

 **Resolving gel stock (12%)** 6 ml 30% A/B 1,5 month! fridge

 3,75 ml of 1,5M Tris-HCl ph8,8

 150 µl 10% SDS

 5,03 ml milliQ H2O

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 dH2O, 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 dH2O
* Wash 10 min
* Discard dH2O, 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 dH2O a few times
* Leave to de-stain in dH2O ON