*MBL Protocol                                                               2023 Barbora Hrnčířová*

**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 H2O (pH adjusted with 5M HCl)

 **1,5M** **Tris-HCl pH8,8** – 18,171g Tris base/100ml miliQ H2O (pH adjusted with 5M HCl)

 **10% SDS** – 10g/100ml miliQ H2O

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

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