Poly-Acrylamid Gel Electrophoresis (PAGE)

A SDS PAGE is used to analyze protein samples, for examples after protein purification. You can decide between a continuous or discontinuous PAGE.

Aim of the Experiment

Materials

- *dd* H_2O
- Precast continuous gel (Mini Proteas TGX gels, 8-16 %, Bio-Rad, USA)
- Loading dye (2x Laemmli, Sigma Aldrich, USA)
- Protein ladder (Color Prestained Protein Standard, Broad Range, NEB, USA)
- 40 % Acrylamide (Rotiphorese Gel 40 (29:1), Carl Roth, Germany)
- SDS running buffer (Carl Roth, Germany)
- 10 % Ammonium persulfate (APS, Thermo Fisher Scientific, USA)
- 10 % Sodium dodecyl sulfate (Carl Roth, Germany)
- Tetramethylethylenediamine (TEMED, Carl Roth, Germany)
- Gel chamber (Mini Protean System, Bio-Rad, USA)
- Protein stain (Roti-Blue Quick, Carl Roth, Germany)
- Electrophoresis power source (VWR, Germany)
- UV detector (Peqlab, Germany)
- Separating (table **??**) and Stacking gel (table **??**): Prepare in this order. After pouring the separating gel overlay the solution with isopropanol, which helps to get a flat surface. Remove the isopropanol after polymerization of the separating gel and before pouring the stacking gel. Then add an appropriate comb. You can store the final gel in SDS running buffer at 4 °C at least for 2 weeks.

Table 1: 10 % Separating gel

Volume [ml]	**Chemicals**
4.83	*dd* H_2O
2.5	Tris-HCl (1.5 M) [pH 8.8]
2.5	Acrylamide (40 %)
0.1	SDS (10 %)
0.05	APS (10 %)
0.02	TEMED

Table 2: Stacking gel

Volume [ml]	**Chemicals**
2.8	*dd* H_2O
1.3	Tris-HCl (0.5 M) [pH 6.8]
0.85	Acrylamide (40 %)
0.05	SDS (10 %)
0.028	APS (10 %)
0.008	TEMED

Procedure

1. Insert the gel in the running chamber and fill it with 1x SDS running buffer.
2. Mix samples with loading buffer and heat them up to 95 °C for 5 min before loading them on the gel. Also load 5 µl of the Protein ladder.
3. Run the gel at 250 V for 45 min.
4. Let the gel incubate in a protein stain for at least 30 min.
5. Image gel under a visible light source.