## SDS-Page

- For each gel (12.5 %) aliquot:
  - ◊ 0.8 mL Tris-HCL (1.88 M), pH = 8.8
  - ◊ 0.8 mL SDS (0.5 %)
  - ◊ 1.67 mL Acryl-/Bisacrylamid (5:1)
  - ◊ 0.73 mL $H_2O$
  - ◊ 2.7 µL TEMED (99 %)
  - ◊ 20 µL APS (10%)
- Pour the solution quickly into the gel casting form. Leave about 2 centimeters below the bottom of the comb for the stacking gel.
- Layer isopropanol on top of the gel.
- Leave the separating gel at room temperature for >60 minutes to polymerize.
- Remove isopropanol and wash surface of the separating gel with $H_2O$. Wait until the surface is dry.
- For each stacking gel (5 %) aliquot:
  - ◊ 235 µL Tris-HCL (0.625 M), pH = 6.8
  - ◊ 235 µL SDS (0.5 %)
  - ◊ 195 µL Acryl-/Bisacrylamid (5:1)
  - ◊ 335 µL $H_2O$
  - ◊ 1.2 µL TEMED (99 %)
  - ◊ 6 µL APS (10 %)

- Insert comb without getting bubbles stuck underneath.
- Leave the gel at room temperature for > 60 minutes to polymerize.
- For storage:
    - ◊ Remove sealing and store the gel wrapped in moistened paper towel at 4 °C.
- Preparing the sample:
    - ◊ Mix your protein mixture 3:1 with PBJR buffer (15 µL protein solution + 5 µL PBJR buffer).
    - ◊ Heat for 5 minutes at 95 °C.
- Running the gel:
    - ◊ Remove sealing, put the polymerized gel into gel box and pour SDS-PAGE running buffer into the negative and positive electrode chamber.
    - ◊ Remove comp without destroying the gel pockets.
    - ◊ Pipet the SDS running buffer in the gel pockets up and down for flushing the gel pockets.
    - ◊ Pipet slowly 20 µL of the sample into the gel pockets.
    - ◊ Make sure to include at least one lane with molecular weight standards to determinate the molecular weight of the sample.
    - ◊ Connect the power lead and run the stacking gel with 10 mA until the blue dye front enters the separating gel.
    - ◊ When the distance of the lowest molecular weight standard lane to the gel end is down to 0.5 cm stop the electrophoresis by turning off the power supply.
- Staining the polyacrylamide gel (Colloidal Coomassie Brilliant Blue staining):
    - ◊ After finishing the SDS-PAGE remove gel from gel casting form and transfer it into a box.
    - ◊ Add 100 mL of the Colloidal Coomassie Brilliant Blue staining solution to your polyacrylamide gel.
    - ◊ Incubate the gel in the solution at room temperature until the protein bands got an intensive blue color. Shake the gel continuously during incubation.
    - ◊ Remove the staining solution.

- ◊ Wash the gel with 7 % (v/v) acetic acid in $H_2O$ for decoloration.
- ◊ Incubate the gel in $H_2O$ (2-6 h) for bleaching the background. Shake the gel continuously during incubation. If necessary, replace the colored water with new one.

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