SDS PAGE

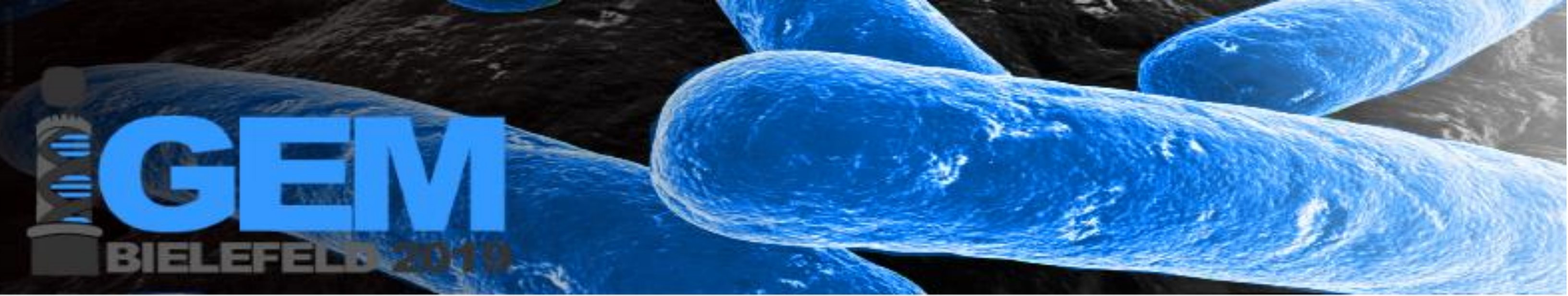
- For each gel (12.5 %) aliquot:
  - 0.8 mL Tris-HCL (1.88 M), pH = 8.8
  - 0.8 mL SDS (0.5 %)
  - 1.67 mL Acryl-/Bisacrylamid (5:1)
  - 0.73 mL H2O
  - 2.7 µL TEMED (99 %)
  - 20 µL APS (10%)
  - Pour the solution quickly into the gel casting form. Leave about 2 centimeters below the bottom of the comb 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 H2O. 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 H2O
  - 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 H2O for decoloration.
- Incubate the gel in H2O (2-6 h) for bleaching the background. Shake the gel continuously during incubation. If necessary, replace the colored water with new one.

From: iGEM Bielefeld-CeBiTec

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