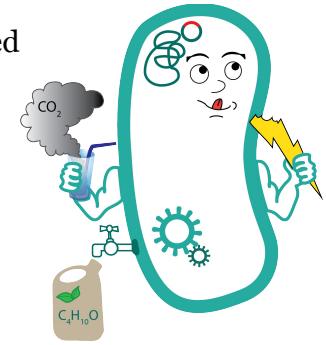
SDS-Page

- Pouring the polyacrylamide gel:
  - For each separating gel (12 %) aliquote:
    - .35 ml Bisacrylamid/Acrylamid (0.8 % , 30 %, at the ratio of 37.5:1)
    - 0.675 ml H_2O
    - 0.675 ml 1.88 M Tris-HCl (pH 8.8)
    - 0.675 ml 0.5 % SDS
  - Add 120 µl 10 % ammonium persulfate and 37,5 µl TEMED to each aliquote and mix
  - 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 the surface of the separating gel with H_2O. Wait until the surface is dry
  - For each stacking gel (5 %) aliquote:
    - 0.309 ml Bisacrylamid/Acrylamid (0.8 % , 30 %, at the ratio of 37.5:1)
    - 0.726 ml H_2O
    - 0.375 ml 0.625 M Tris-HCl (pH 6.8)
    - 0.375 ml 0.5 % SDS
  - Add 60 µl 10 % ammonium persulfate and 22,5 µl TEMED to each aliquote and mix
  - 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 (PageRuler Prestained Protein Ladder™ (Fa. Fermentas)) 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
  - Raise amperage up to 20 mA for running 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 Coomaassie Brilliant Blue staining solution to your polyacrylamid 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