# SDS-Page

For four mini-gels:

| | Stacking Gel (3 %) | Separating Gel (10 %) |
|---|---|---|
| 1.5 M Tris-HCl pH 8.8 | – | 5 ml |
| 0.5 M Tris-HCl pH 6.8 | 2.5 ml | – |
| 30 % Acrylamide | 1.1 ml | 8 ml |
| Water | 6.1 ml | 6.8 ml |
| 20 %SDS | 50 µl | 100 µl |
| 0.5 M EDTA pH 8 | 50 µl | 100 µl |
| APS (100 mg/ml) | 200 µl | 200 µl |
| TEMED | 15 µl | 8 µl |
| Final | 10 ml | 20 ml |

Work with gloves (unpolymerized acrylamide is toxic and keratin gives contaminating bands)!

1. Wash glass plates thoroughly with sponge and soap, rinse well with VE water and wipe with 70% ethanol. Assemble glass plates into gel pouring device.
2. Prepare solutions for 4x separation and 4x stacking gels in 50-ml Falcons (don't add APS and TEMED).
3. Add APS und TEMED to separating gel solution and mix well.
4. Pour gel solution between the glass plates in the pouring device and overlay with isopropanol using a Pasteur pipette. Let the gel polymerize for 45 - 60 min.
5. Decant isopropanol and rinse gels thoroughly with distilled water and remove remaining water on top of the gel with Whatman paper (avoid touching the gel while doing this!).
6. Add APS and TEMED to the stacking gel solution and mix well.
7. Pour solution onto the separating gel and slide-in combs; avoid bubbles!
8. Let the gel polymerize for 30 - 45 min.
9. Carefully remove combs and mark the pockets on the outer glass plate.
10. Assemble glass plates with gels into the running device and place it into the running chamber.
11. Fill-in electrode buffer into the interior chamber until the buffer level is between outer and inner glass plates; pour remaining buffer into the outer chamber.
12. Load the calculated volume of the samples on the gels using a 20-µl pipette and load 3 µl of protein marker (M). Prepare one set with the lower and one with the higher protein amount (not for screening gels).

13. Conduct electrophoresis at 120 V for ~ 60 min until the chlorophyll is just about to exit the gel; in the meanwhile, prepare Whatman paper and nitrocellulose membranes for blotting.