Protein gel electrophoresis (Lämmli)

- solutions:

-Tris gel buffer:
375 mM Tris pH 8.8
0.1% SDS

-stacking gel (4%):
26.67 ml acrylamide-stock (30%)
0.2 g SDS (0.1%)
3 g Tris base
adjust pH to 6.8
add H_2O to 200 ml
store at 4°C

-10x running buffer:

250 mM	Tris	30.3 g/l Tris base
1920 mM	glycine	144.2 g/l
1%	SDS	10 g/l

pH should be ~8.3 (do not adjust pH!!!)

-3x Lämmli:
187.5 mM Tris pH 6.8
6% SDS
30% glycerol
15% β-mercaptoethanol
0.003% bromphenolblue
make 1 ml aliquots
heat 5 min at 100°C in heating block

-sample preparation:
x µl protein extract
5 µl 3x Lämmli
H_2O to 15 µl
-denature 5 min at 95-100°C (heating block)
-samples can be stored at –20°C (heat again before loading)

-gel preparation:
-for Hoefer Minigels (Mighty Small II SE250)
-clean 1 glass plate, 1 white plate and 2 spacers (0.75 mm) per gel
-assemble for pouring (white plate in the back, do not tighten screws too much, make sure spacers and plates are level at the bottom)
-prepare 10% APS (ammonium persulfate) in H_2O
-make a mark on glass plate to indicate the volume of the gel (~1 cm below the comb)
-acrylamide-solution: e.g. for two 10% gels:
3.3 ml acrylamide-stock (30%)
6.7 ml Tris gel buffer
200 µl 10% APS
6 µl TEMED
-mix well and pour gel up to mark
-add 200 µl n-butanol on top
-polymerize for ~1 h

-remove gel from pouring unit, remove n-butanol with H_2O
-remove drops between plates using a whatman paper
-stacking gel: for 2 gels:

4 ml stacking gel (4%)
200 µl 10% APS
4 µl TEMED

-mix well and fill gel up to the very top
-immediately add combs (stacking gel polymerize very fast)
-polymerize for 15 min

-gel run:
-prepare 250 ml 1x running buffer
-carefully remove combs and load samples
-add protein standard
-run gels for 50-60 min at 25-30 mA per gel (use water cooling and mix buffer) until bromphenolblue reaches bottom of gel and runs out
-the protein gels can be stained directly or used in a western blot procedure

Staining of protein gels with Coomassie

- solutions:
  - -Coomassie-solution 1: 0.025% Coomassie Brilliant Blue R250, 25% isopropanol, 10% HAc
  - -Coomassie-solution 2: 0.0025% Coomassie Brilliant Blue R250, 10% isopropanol, 10% HAc
  - -destaining solution: 10% isopropanol, 10% HAc

-shake protein gel 20-30 min in Coomassie-solution 1
-partially destain in Coomassie-solution 2 (gels can be left in this solution almost indefinitely)
-destain 2-3 h in destaining solution (add a rolled kim wipe to absorb the coomassie)
-fix for ~1 h in 10% HAc
-sandwich gel between wet cellophane foil, put sandwich on a whatman filter and cover with saran wrap
-dry for 1 h on gel drier at 80°C
-make sure gels do not curl