

SDS-PAGE

Method:

1. Assemble gel apparatus - two side spacers, one bottom spacer, two glass pieces; be sure to have the small piece of glass fit snugly against the pads on the spacers or buffer will flow through.
2. Melt 1% agar and seal the sides of the clamped apparatus.
3. Pour ~ 40 ml of the running gel (lower) mixture into the apparatus; add dW on top to keep meniscus flat and keep air bubbles from entering; allow 20-30 minutes to polymerize; drain water. Leave room for teeth of comb + 1 cm. (Can overlay with a pastuer pipette 0.1% SDS for gels with <8% acrylamide; isobutanol > 10% acryl).
4. Pour ~15 ml of stacking gel mix on top and add comb; allow 20 minutes to polymerize.
5. Pour diluted running buffer in basin of apparatus holder, remove bottom spacer and clamps from apparatus; clamp gel into holder; fill top basin. Be sure there are no air bubbles trapped at the bottom of the apparatus (where the bottom spacer goes).
6. Add 30 ul of eluent sample to 30 ul of sample buffer (2% BME), boil for 5 minutes with lids clamped down and apply to each well of the column. Standard
7. Connect to power supply and run at either: (1) constant current 60 mA for 3 hr run or; (2) 40 mA for 5hr or; (3) constant voltage - 40 V for ON run.
8. After use clean combs and spacers with water and EtOH.

Recipe:

Running gel (60 ml)

- ml 1.5 M Tris pH 8.8
- ul 20% SDS
- ml 30% Acrylamide/Bis mix (37.5:1 Ultrapure Protogel - on bench) - for a 7.5% gel
- ml dW
- ul TEMED (N,N,N',N' - tetramethylethylenediamine) - last step; swirl flask quickly
- ul 10% ammonium persulfate

Stacking gel (15 ml)

- 0.5M Tris pH 6.8
- ul 20% SDS
- ml 30% Acrylamide/Bis
- ml dW
- ul TEMED
- ul 10% AP

10X SDS Running buffer (pH 8.3)

- g Tris
- g glycine
- ml dW
- ml 10% w/v stock SDS
- to 1000 with dW

2X Laemelli Sample buffer

- mM Tris-Cl (pH 6.8)
- SDS (electrophoresis grade)
- bromophenol blue
- glycerol
- Add 500 ul of LSB to 50 ul of BME and add equal volume of sample to achieve 1X

Notes:

Proteins purified for sequencing require purer SDS, for methods see Methods Enz. 91:227 (1983).