Preparing samples for SDS-PAGE

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- Bacterial culture
- Antibiotics
- IPTG
- Spectrophotometer
- 1,5 ul cuvettes
- 1x SDS loading buffer

Procedure

1. The day before: make an overnight culture(s) of desired bacteria with appropriate antibiotics.
2. In the morning: refresh the o/n culture(s): 100 ul o/n culture to 2 ml fresh LB
3. Incubate until OD reaches 0,5. Add IPTG: final concentration should be 0,5 mM.

   For 2100 ul culture add 0,5 ul IPTG (stock concentration 500 mg/ml)
4. Incubate in 30 C for 4h.
5. Spin down the cells and resuspend the pellet in 50 µl of 1xSDS sample buffer (with DTT).
6. Incubate the samples in 95 C for 10 minutes. After boiling spin them at max. speed for 10 minutes.
7. Take 8 µl from the very top of the liquid for SDS-PAGE analysis.

SDS-PAGE

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Materials

- 10 % APS (ammonium persulfate)
- TEMED
- 40 % acrylamide
- 4 x separating buffer (1,5 M Tris-HCl pH 8.8, 0.4 % SDS)
- 2x Stacking gel (0.25 M Tris-HCl pH 6.8, 0.2 % SDS)
- sterile water

Procedure

Casting the gel

1. Check that the glasses are clean. Wash and rinse with water and ethanol if necessary.
2. Set up the casting gear. Press glasses against each other so that the lower end is even (check by placing on the table for example). You may check with water that the set-up doesn't leak. Remove the water by pouring and using Whatman filter paper.
3. Mark the border of separating and stacking gel by measuring 0,5 cm below the comb.
4. Mix the separating gel according to the table below. The mixture is enough for two gels. APs and TEMED are responsible for the polymerization so add these last.
5. Pour or pipette the mixture