SDS-Page

Protocol for running an SDS-Page

Day 1

N.B.: Keep everything on ice until loaded on the gel.

- Transfer 2 mL from each culture sample into a 2 mL centrifuge tube and spin for 5 min at 13.000 rpm and 4°C.
- Remove supernatant.
- Re-suspend the pellets in 500 uL Lysis Buffer.
- Sonicate each sample for ~10-15 sec.
- Spin each sample for 5 min at 13.000 rpm and 4°C.
- For each sample mix 10 uL loading dye with 50 uL sample.
- Fill gel tub with Tris/Glycine/SDS buffer
- Load gels into pre-cast SDS-gel (Criterion TGX Stain-Free Precast Gel).
- Run for ~15-30 min at 250 V.
- Take out the gel and place it in the Bio-Rad Universal Hood iii and image it using Image Lab software.

Buffers

Lysis buffer

- 5.0 mM Imidazole
- 0.5 M NaCl
- 20 mM Tris-HCl pH 7.9
- 1.0 mM PMSF

For 15 mL mix:

- 18.85 uL 4M Imidazole
- 1.5 mL 5 M NaCl
- 0.6 mL 0.5 M Tris-HCl pH 7.9

- 150 uL 0.1 M PMSF