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Preparation of bacterial cell lysate for SDS-PAGE (2022 iGEM)

Forked from Preparation of bacterial cell lysate for proteomics (LC-MS) by freeze and thaw cycles

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ABSTRACT

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FORK NOTE

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Preparation of bacterial cell lysate for SDS-PAGE

1 Grow the cells overnight, to an OD_{600} above 1. If IPTG induction needed, at it around OD_{600} 0.2-0.3 (keep IPTG stock solution at -20 freezer).

16h

2 ⚠

Take one 1 ml of OD_{600} = 1 from each sample. Correct the sample volume to obtain an equivalent size pellet. For us, as quantified using NanoCym950 nanoparticles (equivalent to the size of *E. coli*), 1 OD600 equals to 10^8 nanoparticles per mL. We estimate each well on SDS-PAGE contains proteins from 2*10^6 bacteria.

10m

3 Spin down the bacterial cells for 1 min at 13,000 rpm in a microfuge.

1m

4 Aspirate any trace of supernatant with a vacuum line avoiding touching the pellet, but removing all liquid.

1m

5 To each cell pellet, add 250 µL of distilled water, resuspend the pellet. Add 250 µL 2x SDS sample buffer (see below). Heat with a 95 degree heating block for 10 minutes. Store the samples at -20 degree freezer until SDS-PAGE.

Receipt

6 2x SDS sample buffer, works for Tris-Cl or MOPS based gels

- Glycerol 20 ml
- SDS 4 g
- 0.5 M Tris-HCl pH 6.8 stock solution 25 ml
- Bromo Phenol Blue 1 mg; dissolve and volume to 95 ml
- add 5% β-Mercaptoethanol before usage

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