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

Alexandro Rodríguez-Rojas[1]

[1]Institute of Biology, Freie Universität Berlin

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Alexandro Rodriguez-Rojas

alexrrus

ABSTRACT

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Preparation of bacterial cell lysate for proteomics (LC-MS) by freeze and thaw cycles (developed by Alexandro Rodríguez-Rojas, FU Berlin, a.rojas@fu-berlin.de).

1 Grow the cells to an OD_{600} of 0.4 (six tube per condition including control groups). Treat the cells with the desired conditions (typically 30 minutes).

2 Take one 1 ml from each sample. Correct the sample volume to obtain an equivalent size pellet.

3 Spin down the bacterial cells for 3 min at 10 000 g in a microfuge.

Extract any trace of supernatant by aspiration with a vacuum line avoiding touching the pellet but removing all liquid.

4

5 To each cell pellet, add 50 µl of the urea denaturing buffer (see below)

6 Freeze quickly on dry ice or liquid nitrogen and leave for at least 2 min. Thaw immediately at 37°C in a nearby water bath. Mix well by flicking the tubes and make sure that urea is well re-dissolved.

7 Repeat the two previous steps for four more cycles (5 freeze-thaw-mixing cycles in all).

8 Use 25 µl of lysate for downstream proteomic analysis and preserve the rest as backup. This volume contains approximately between 40 to 60 µg of total protein. Lysis becomes evident when the bacterial suspensions turn from translucid to transparent

Notes

9 1. Urea denaturing buffer:
-6 M urea
- 2 M thiourea
-10 mM HEPES (pH 8.0).

2. This protocol was established for *Escherichia coli* MG1655. It has been tested for other bacterial species such as *Pseudomonas aeruginosa* PA14 and *Staphylococcus aureus* SH100. For other bacterial species or strains the number of freeze- thaw cycles could change.

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