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B-PER Lysis--CHEM 384

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We are still developing and optimizing this protocol

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Abstract

The Thermo Scientific B-PER Bacterial Protein Extraction Reagent enables mild extraction of proteins from bacteria (*E. coli*) without the need for mechanical disruption. The reagent may be used for soluble protein extraction and inclusion body purification from bacterial cell lysates. The B-PER Reagent with Enzymes is supplied with lysozyme and DNase I to improve the extraction efficiency of large (> 70 kDa) molecular weight proteins and proteins expressed in inclusion bodies.

Guidelines

Important Product Information

The B-PER Reagent extracts proteins from recently prepared cells and frozen cells. Extraction is typically more effective with frozen cells. The amount of reagent required depends on the amount of cell paste.

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B-PER Reagent effectively extracts soluble proteins from several common bacterial host strains and is especially suitable for the protease-defective expression host BL21 strains. If lysis is inefficient for a particular bacterial strain, freeze cells before extraction.

Troubleshooting

B-PER Lysis to extract soluble protein

1 Pellet bacterial cells by centrifugation at 5000 × g in a tared tube for 00:10:00 .

2 Decant your spent LB and determine the mass of your cell pellet.

3 Freeze your pellet in the -20°C freezer overnight or quickly freeze your pellet in liquid nitrogen. When you are ready to proceed, thaw your cells at room temperature.

4 Add 4 mL of B-PER Reagent per gram of wet cell pellet. Pipette the suspension up and down until it is homogeneous.

5 Optional: Add 2 µL of lysozyme and 2 µL of DNase I per 1 mL of B-PER Reagent added.

6 Incubate 00:15:00 at room temperature.

7 Centrifuge lysate at high speed (~15,000 × g) for 00:05:00 to separate soluble proteins from the insoluble proteins.

Note

Note: If a large percentage of over-expressed protein remains in the pellet, the protein of interest might be expressed in inclusion bodies.