NZY Bacterial Cell Lysis Buffer

Catalogue number:

MB17801, 250 mL
MB17802, 2 × 250 mL

Features

- Disrupt cells without mechanical procedures
- Ready-to-use formulation
- Ideal for high-throughput (HTP) methods
- DNase I and Lysozyme provided separately
- Outstanding yields of extracted proteins

Description

NZY Bacterial Cell Lysis Buffer is an innovative product developed at NZYTech for the gentle disruption of *Escherichia coli* cell wall, generating a homogeneous cell-free extract. It provides a rapid and cost-effective alternative to harsh chemicals or mechanical procedures, such as French Press or sonication, for releasing recombinant and native proteins without denaturation. This extraction reagent is a Tris-buffered formulation (pH 7.5) with lysozyme and DNase I provided separately. The addition of these enzymes allows a most efficient extraction. However, for some over-expressed proteins and particular *E. coli* strains the addition of lysozyme may not be required. Please consider that the presence of lysozyme and DNase I might interfere with some downstream applications (in these situations, do not add the enzymes).

Additional components, such as protease inhibitors, salts, reducing agents and chelating agents (not provided), may be added to the lysate obtained using the NZY Bacterial Cell Lysis Buffer, depending on the particular application. Note that chelating agents should not be added to the lysate in the presence of lysozyme and DNase I.

Shipping conditions

NZY Bacterial Cell Lysis Buffer, Lysozyme and DNase I are shipped at 4 ^{o}C to room temperature.

Storage conditions

Store NZY Bacterial Cell Lysis at room temperature and lysozyme and DNase I at -20 °C in a freezer without defrost cycles. Buffer and enzymes are stable until their expiration dates if stored and handled properly.

System Components

Component	MB17801	MB17802
NZY Bacterial Cell Lysis buffer	250 mL	5 x 250 mL
Lysozyme (at 50 mg/mL)	0.5 mL	2 x 0.5 mL
DNase I (at 2 mg/mL)	0.5 mL	2 x 0.5 mL

Protocol for extracting recombinant or native proteins from bacteria (*E. coli*)

1. Harvest cells from liquid culture by centrifugation at 2000-5000 ×*g* for 10 min at 4 °C.

2. Decant and allow the pellet to drain, removing as much supernatant as possible. Determine the weight of the pellet. **Note:** The protein extraction is typically more efficient if the pellets are frozen for at least 30 min.

3. Resuspend the cell pellet at room temperature by pipetting or gentle vortexing using 5 mL of NZY Bacterial Cell Lysis Buffer per gram of cell past.
Note: In high-throughput protocols, when using small cultures, typically use 1 mL of Buffer per 5-mL cultures.

4. Optional but highly recommended: Add 2 µL of lysozyme at 50 mg/mL and 2 µL of DNase I per 1 mL of NZY Bacterial Cell Lysis Buffer. Optional: Add EDTA-free protease inhibitors (not provided).

5. Incubate the cell suspension on a shaking platform or rotating mixer for 10-20 min at room temperature.

6. Remove insoluble cell debris by centrifugation at 15,000 ×*g* for 15 min at 4 °C.
Note: If desired, save the pellet for inclusion body purification.

Important notes

- NZY Bacterial Cell Lysis Buffer can be used on fresh or frozen cell pellets. Superior extraction efficiencies can be obtained by freezing the pellets before adding the Buffer.
- Extraction efficiency can be strain-dependent. The NZY Bacterial Cell Lysis Buffer can be used to disrupt the most common bacterial host strains and it is especially efficient with BL21 strain and its derivatives. The use of pLysS or pLysE hosts enhances the extraction procedure.
- Whole cell lysates from NZY Bacterial Cell Lysis Buffer are compatible with Bradford protein assay as well as with Lowry and biuret reagents.
- The resulting protein extract can be used in preparation of different purification methods, such as GST or His-tagged immobilized metal affinity chromatography (IMAC), or in other applications.

Quality control assays

Functional assay

NZY Bacterial Cell Lysis Buffer is tested functionally in a protocol for extracting recombinant proteins from *E. coli* strain BL21(DE3). The released recombinant proteins are purified through IMAC and separated through SDS-PAGE.

Data

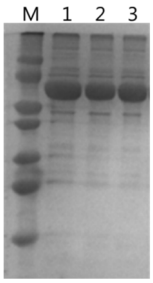

Figure 1. Comparing the efficiency of cell lysis using three different protein extraction methods. *E. coli* cells harvested from 5 mL of cultured media were lysed using three different procedures (Lane 1: NZY Bacterial Cell Lysis Buffer; Lane 2: Sonication; Lane 3: Competing product). The recombinant protein was purified through IMAC and separated through SDS-PAGE. M: Low Molecular Weight (LMW) Protein Marker (MB082).

Troubleshooting

The cell lysate is hazy
• Cell density is too high
Additional NZY Bacterial Cell Lysis Buffer can be added for high density cell cultures to solubilize remaining particulates. Cell lysate can be clarified by centrifugation at 16,000 ×*g* for 20 min.
• Incubation time is too short
Incubate the cell lysate for at least 20 min to ensure that all cell components are completely solubilized.
• Temperature is too low
Incubate lysate at room temperature (20-25 °C). When using frozen pellets extend the incubation period or quickly warm lysate to room temperature.

Viscosity of extract is high
• DNase I has low activity or is inactive Cell density is too high
Add more DNase I. Check if the enzyme is stored and handled properly.

Protein of interest is not solubilized
• Protein of interest is expressed in inclusion bodies
Adjust expression conditions. Ensure that lysozyme and DNase I are added during cell lysis.
• Cells are not completely lysed
Ensure cell lysis is performed for at least 20 min at room temperature to allow for complete lysis of the cells.

Certificate of Analysis

Test	Result
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

For research use only.

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genes & enzymes

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