

**Easy BacLysis Protein Extraction Solution
Plus Hercules Endonuclease**

Cat. No. L00240



Technical Manual No. TM0238

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I. DESCRIPTION

The Easy BacLysis Protein Extraction Solution facilitates the removal of soluble proteins from *E. coli* by gently disrupting the bacterial cell wall. This precisely formulated product provides a simple, speedy, low-cost alternative to mechanical methods such as French Press or sonication. The proprietary formulation employs a mixture of non-ionic detergents that perforate the cell wall without denaturing soluble protein. Depending on the particular intended application, additional components, such as lysozyme, protease inhibitors, salts, reducing agents, or chelating agents, may be added to the buffer. The buffer may be used for both soluble protein extraction and inclusion body purification from total bacterial cell lysates.

Easy BacLysis Protein Extraction Solution Plus Hercules Endonuclease also gently releases target proteins from *E. coli*. It also markedly reduces extract viscosity prior to downstream processing. Cells are harvested by centrifugation and then suspended in BacLysis Solution at room temperature. Hercules Endonuclease is then added. During a brief incubation, proteins are released and nucleic acids are digested. Insoluble protein and cell debris are easily removed by centrifugation. The resulting low-viscosity clarified extract is then ready for purification and analysis. The extract is compatible with most chromatography resins. The insoluble fraction can be further processed to yield purified inclusion bodies.

II. KEY FEATURES

- Easy-to-use, single-solution format
- Rapid cell lysis of recombinant *E. coli* is complete in just 20 minutes
- Flexible format, compatible with small- and large-scale protein extraction
- Designed for both soluble and insoluble protein extraction

- Purifies inclusion bodies to near-homogenous levels (does not solubilize inclusion bodies)

III. APPLICATIONS

- Disruption of bacterial cells for the removal of active proteins
- Inclusion body purification

IV. COMPONENTS

Components	L00230	L00240
Easy BacLysis Protein Extraction Solution	500 ml	500 ml
Hercules Endonuclease		25 kU

V. STORAGE

Store Easy BacLysis Protein Extraction Solution at 4°C. Hercules Endonuclease is supplied in 50% glycerol containing 10 mM Tris-HCl, 20 mM NaCl, and 2 mM MgCl₂, pH 8.0. The enzyme is stored at -20°C. **DO NOT** store at -70°C. Freezing Hercules Endonuclease results in loss of activity.

VI. GENERAL CONSIDERATIONS

1. Easy BacLysis Protein Extraction Solution and Hercules Endonuclease are most efficient when used at room temperature. Storage of Easy BacLysis Protein Extraction Solution at temperatures below 4°C may cause precipitation of the detergents. To redissolve any precipitants, incubate in a room temperature water bath with gentle swirling.
2. Easy BacLysis Protein Extraction Solution extracts soluble proteins and most inclusion bodies from bacteria. Although the protocols provided here are optimized for extraction of both soluble and insoluble (inclusion body) proteins, it is often necessary to perform a mini-scale extraction to determine the solubility of the specific recombinant protein before performing a large-scale protein extraction and purification.
3. Easy BacLysis Protein Extraction Solution is capable of extracting proteins from both fresh and frozen cells. However, the extraction is typically more effective with frozen cells.
4. Easy BacLysis Protein Extraction Solution is especially suitable for the commonly used, protease-defective bacterial expression host BL21 strains. Freezing the bacterial cells prior to extraction may increase efficiency in other strains.
5. Easy BacLysis Protein Extraction Solution is supplied in a Tris-based buffer system. It is compatible with affinity supports such as GST•Bind™, GST•Mag™, His•Bind®, His•Mag™, and S•Tag™ purification resin, and other chromatography matrices.
6. Hercules Endonuclease loses approximately 50% of its relative activity when monovalent cation concentrations exceed 50 mM, phosphate concentrations exceed 20 mM, or ammonium sulfate concentrations exceed 25 mM.

VII. GENERAL PROTOCOL USING EASY BACLYSIS PROTEIN EXTRACTION

A. Soluble Fraction

1. Pellet bacterial cells by centrifugation at 8,000 rpm for 10 minutes using a weighed centrifuge tube. For small-scale extractions (1.5 ml or less), centrifugation can be performed in a 1.5-ml tube at 8,000 rpm for 3 minutes. Remove as much liquid as possible.
2. Resuspend the cell pellet in room-temperature Easy BacLysis Protein Extraction Solution by pipetting or gentle vortexing. This typically corresponds to about 5 ml per 50 ml culture and 20 ml per 250 ml culture. For small cultures, use up to 1/5 culture volume for resuspension (For example, use 300 μ l Easy BacLysis Protein Extraction Solution for 1.5 ml cultures.). There are no adverse effects to using higher volumes of Easy BacLysis Protein Extraction Solution.

Optional:

- a) To reduce viscosity of the lysate, add 25 U Hercules Endonuclease per ml Easy BacLysis Protein Extraction Solution adjusted for resuspension.
- b) To improve efficiency of the extract in non-pLysS and -pLysE hosts, add 1 kU Lysozyme solution per 1 ml Easy BacLysis Protein Extraction Solution.

Add protease inhibitors. Protease inhibitors are compatible with Easy BacLysis Protein Extraction Solution, Hercules Endonuclease, and Lysozyme. Although purification may remove active inhibitors, dialysis, or gel filtration is recommended prior to cleavage.

3. Incubate the cell suspension on a shaking platform or rotating mixer at a slow setting for 10 to 20 minutes at room temperature.
4. Remove insoluble cell debris by centrifugation at 12,000 rpm for 20 minutes at 4°C. If desired, save the pellet for inclusion body purification.
5. Transfer the supernatant to a fresh tube. The soluble extract can be loaded directly onto any purification resin. Maintain clarified extracts on ice for short term storage (2 to 3 hours) or freeze at -20°C until needed. Extracts should be stored at a temperature compatible with target protein activity; some target proteins may be inactivated by freeze-thaw cycles.

B. Inclusion Body Purification

1. Process the induced culture according to steps 1-4 above for the soluble protein fraction.
2. Resuspend the pellet from step 4 above in the same volume of Easy BacLysis Protein Extraction Solution that was used to resuspend the original cell pellet. Pipette up and down and vortex to obtain an even suspension.

Complete resuspension of the pellet will solubilize and remove contaminating proteins. This is critical to obtaining a high purity preparation.

3. Add lysozyme to a final concentration of 1 kU/ml. Mix by gently vortexing and incubate at room temperature for 5 minutes.
4. Add five volumes of 1:10 diluted Easy BacLysis Protein Extraction Solution to the suspension. Mix by vortexing.
5. Collect the inclusion bodies by centrifugation at 12,000 rpm for 15 minutes. Resuspend the pellet in five volumes of 1:10 diluted Easy BacLysis Protein Extraction Solution.
6. Repeat Step 5 two more times.
7. Dissolve the purified inclusion bodies in denaturing agents and proceed to further refolding or purification procedures.

VIII. ORDER INFORMATION

Easy BacLysis Protein Extraction Solution Cat. No. L00230

Easy BacLysis Protein Extraction Solution Plus Hercules Endonuclease Cat. No. L00240

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