

B-PER[®] Bacterial Protein Extraction Reagent

78243 78248

0736w

Product Description

Number

78243

78248

Description

B-PER[®] Bacterial Protein Extraction Reagent, 165 ml**B-PER[®] Bacterial Protein Extraction Reagent, 500 ml**

Store product at room temperature. Product is shipped at ambient temperature.

Product is guaranteed for one year from the date of purchase if stored and used properly.

Introduction

B-PER[®] Bacterial Protein Extraction Reagent was developed for extraction of proteins, especially recombinant proteins, from bacteria (*E. coli*). The B-PER[®] Reagent has also been used for the extraction of recombinant proteins from insect cells infected by baculovirus. This reagent utilizes a proprietary, mild, nonionic detergent in 20 mM Tris•HCl (pH 7.5) for lysis of the bacterial cells. This method eliminates the need for mechanical disruption utilizing equipment that may not be available in some research laboratories. The novel composition of this reagent provides versatility for different applications and eliminates exogenous contamination of the recombinant protein by the lysis reagent. Depending on the particular application, additional components such as lysozyme, protease inhibitors, salts, reducing agents, chelating agents, etc., may be added to the reagent. The reagent may be used for both soluble protein extraction and inclusion body purification from total bacterial cell lysates.

General Considerations

- Soluble Proteins and Inclusion Bodies:** Recombinant proteins expressed in bacteria often form inclusion bodies, especially when they are expressed at high levels. It is not known exactly how these inclusion bodies are formed, but it is thought that the protein within the inclusion body may be folded incorrectly. Inclusion bodies generally allow greater levels of protein expression and they can be easily separated from a large proportion of bacterial cytoplasmic proteins by centrifugation, providing an effective purification step. The B-PER[®] Reagent effectively extracts soluble proteins and removes most of the soluble protein from the inclusion bodies. Although 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 larger-scale protein extraction and purification.
- Fresh Cells and Frozen Cells:** The B-PER[®] Reagent is capable of extracting proteins from both fresh and frozen cells. However, the extraction is typically more effective with frozen cells.
- Lysozyme:** (Product No. 89833 or 89834) For inclusion body purification, lysozyme should be used to further digest the cell debris and release the inclusion bodies. Lysozyme digestion can significantly improve the purity of inclusion body proteins and will be eliminated during the subsequent washing steps of the protocol.
- E. coli* Strain:** The B-PER[®] Reagent has been tested for use with several common bacterial host strains. It is especially suitable for the commonly used, protease-defective bacterial expression host BL21 strains.¹ If the lysis is not found to be efficient for your particular bacterial strain, freeze the bacterial cells prior to extraction.
- Compatibility:** The B-PER[®] Reagent is supplied in a Tris-based buffer system. Tris•HCl buffer is recommended for subsequent protein purification following protein extraction with the B-PER[®] Reagent.

Telephone: 800-8-PIERCE (800-874-3723) or 815-968-0747 • Fax: 815-968-7316 or 800-842-5007

Internet: <http://www.piercenet.com>

6. **Optional/Supplemental Materials:** Protease inhibitors (Product Nos 78410 or 78415), salts, chelating agents, reducing agents and DNase I (Product No. 89835) may be added directly to the reagent for specific applications.
7. **Protein Extraction from Eukaryotic Cells:** The B-PER[®] Reagent has also been tested for the extraction of recombinant proteins from insect cells infected by baculovirus. The amount of the reagent required depends on the confluence of the infected cells (*see Example Protocols*).

Example Protocols

Additional materials required only for inclusion body purification:

- a. Lysozyme (Product No. 89833 or 89834): Dissolve the lysozyme in B-PER[®] Reagent to a final concentration of 10 mg/ml. A fresh lysozyme solution must be used each time.
- b. Ultrapure Water: Used for 1:10 dilution of the B-PER[®] Reagent to prepare solution for washing inclusion bodies.

Protocol for Mini-Scale Bacterial Protein Extraction (1.5 ml bacterial culture, OD₆₀₀ 1.5-3.0)

1. Pellet bacterial cells by centrifugation at 5,000 RPM for 10 minutes in a microcentrifuge.
Note: The cells can either be used fresh or frozen at -70°C.
2. Resuspend the cells in 300 µl of B-PER[®] Reagent by either vigorously vortexing the mixture or by pipetting up and down until the cell suspension is homogeneous. Vortex for 1 more minute.
3. Centrifuge at 13,000 RPM for 5 minutes to separate the soluble proteins from the insoluble proteins.
4. Collect the supernatant (soluble fraction) and resuspend the pellet (insoluble fraction) in 300 µl of B-PER[®] Reagent. Use 10 µl each of the soluble and insoluble fraction for SDS-PAGE or Western blotting assay to determine the solubility of the recombinant protein. If purification of inclusion bodies is required, proceed to Step 5.
5. For inclusion body purification, add lysozyme (6 µl of 10 mg/ml stock solution) to the resuspended pellet (insoluble fraction generated in Step 4) to a final concentration of 200 µg/ml, and vortex for 1 minute. Add 1 ml of 1:10 diluted B-PER[®] Reagent to the suspension and vortex for 1 minute.
6. Collect inclusion bodies by centrifugation at 13,000 RPM for 10 minutes. Resuspend the pellet in 1 ml of 1:10 diluted B-PER[®] Reagent and vortex for 1 minute.
7. Repeat Step 6 two more times.
8. Resuspend the final inclusion body pellet in 300 µl of ultrapure water or desired buffer. Use 10-20 µl of sample for SDS-PAGE assay to determine the purity of the inclusion body protein.

Protocol for Midi-Scale Bacterial Protein Extraction (40 ml bacterial culture, OD₆₀₀ 1.5-3.0)

1. Pellet bacterial cells by centrifugation at 3,020g (e.g., 5,000 RPM for Beckman JA20 rotor) for 10 minutes.
Note: The cells can either be used fresh or frozen at -70°C.
2. Resuspend the cells in 5 ml of B-PER[®] Reagent either by vortexing or pipetting up and down until the cell suspension is homogeneous. Once a homogeneous mixture is established, shake it gently at room temperature (RT) for 10 minutes.
3. Separate the soluble proteins from the insoluble proteins by centrifugation at 27,200g (e.g., 15,000 RPM for Beckman JA20 rotor) for 15 minutes.
Note: Typically, greater than 90% of the soluble proteins are recovered from this extraction. An additional extraction may increase the yield, but is usually not necessary. The soluble proteins extracted may be used for further purification or analysis. If purification of inclusion bodies is required, proceed to Step 4.
4. For inclusion body purification, add 5 ml of B-PER[®] Reagent to the pellet (insoluble fraction generated in Step 3) and resuspend by vortexing or pipetting up and down.
5. Add lysozyme (100 µl of 10 mg/ml stock solution) into the suspension to a final concentration of 200 µg/ml. Mix well and incubate at RT for 5 minutes. Add 15 ml of 1:10 diluted B-PER[®] Reagent to the suspension. Mix by vortexing.

6. Collect the inclusion bodies by centrifugation at 27,200g for 15 minutes. Resuspend the pellet in 20 ml of 1:10 diluted B-PER[®] Reagent.
7. Repeat Step 6 two more times.
8. Dissolve the purified inclusion bodies in denaturing agents and proceed to further refolding or purification procedures.

Protocol for Maxi-Scale Bacterial Protein Extraction (250 ml bacterial culture, OD₆₀₀ 1.5-3.0)

Note: For larger volume of bacterial cultures, the volume of reagent used should be increased accordingly.

1. Pellet bacterial cells by centrifugation at 3,440g (e.g., 5,000 RPM for Beckman JA 17 rotor) for 10 minutes.

Note: The cells can either be used fresh or frozen at -70°C.

2. Resuspend the cells in 10-20 ml of B-PER[®] Reagent either by vortexing or pipetting up and down until the cell suspension is homogeneous. Once a homogeneous mixture is established, shake it gently at RT for 10 minutes.
3. Separate the soluble proteins from the insoluble proteins by centrifugation at 27,000g (e.g., 14,000 RPM for Beckman JA17 rotor) for 15 minutes.

Note: Typically, greater than 90% of the soluble proteins are recovered from this extraction. An additional extraction may increase the yield but is usually not necessary. The soluble proteins extracted may be used for further purification or analysis. If purification of inclusion bodies is required, proceed to Step 4.

4. For inclusion body purification, add 10-20 ml of B-PER[®] Reagent to the pellet (insoluble fraction generated in Step 3) and resuspend by vortexing or pipetting up and down.
5. Add lysozyme (200-400 µl of 10 mg/ml stock solution) into the suspension to a final concentration of 200 µg/ml. Mix well and incubate at RT for 5 minutes. Add 100 ml of 1:10 diluted B-PER[®] Reagent into the suspension. Mix by vortexing.
6. Collect the inclusion bodies by centrifugation at 27,000g for 15 minutes. Resuspend the pellet in 100 ml 1:10 diluted B-PER[®] Reagent.
7. Repeat Step 6 two more times.

Note: After the final centrifugation, proceed to Step 8 without resuspension.

8. Dissolve the purified inclusion bodies in denaturing agents and proceed to further refolding or purification procedures.

Protocol for Protein Extraction from Insect Cells - Example Method I (Monolayer Culture)

1. Grow and infect insect cells according to standard protocols.
2. Remove (pour off) culture media from a monolayer culture grown in a 100 mm plate. Add 0.5-1 ml of B-PER[®] Reagent.
3. Shake the plate briefly and collect the lysate using a cell scraper. Transfer lysate into a centrifuge tube.
4. Separate the soluble proteins from the insoluble proteins (and cell debris) by centrifugation at 27,000g for 15 minutes.

Protocol for Protein Extraction from Insect Cells - Example Method II

1. Grow and infect insect cells according to standard protocols.
2. Collect cells by low speed centrifugation and pour off liquid.
3. Add 10 ml of B-PER[®] Reagent for every 1 gram of wet cell pellet.
4. Resuspend pellet and shake the suspension for 10 minutes.
5. Separate the soluble proteins from the insoluble proteins (and cell debris) by centrifugation at 27,000g for 15 minutes.

Related Products Available from Pierce

Number	Description
89833, 89834	Lysozyme
89835	DNase I
78410	Halt™ Protease Inhibitor Cocktail Kit
78415	Halt™ Protease Inhibitor Cocktail Kit, EDTA-Free
78100	B-PER® 6xHis Fusion Protein Purification Kit
78200	B-PER® GST Fusion Protein Purification Kit
78300	B-PER® 6xHis Spin Purification Kit
78400	B-PER® GST Spin Purification Kit
23227	BCA Protein Assay Kit
23236	Coomassie® Plus Protein Assay

Reference

1. Phillips, T. A., VanBogelen, R. A. and Neidhardt, F. C. (1984). *J. Bacteriol.* **159**, 283-287.

© Pierce Chemical Company, 8/2001. Printed in the U.S.A.

The Pierce B-PER® Reagent is covered by U.S. Patent No. 6,174,704 B1.