

# B-PER™ Bacterial Protein Extraction Reagent (in phosphate buffer)

78266

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| Number | Description   |
|--------|---|
| 78266  | <b>B-PER Bacterial Protein Extraction Reagent (in phosphate buffer), 500mL</b><br><b>Storage:</b> Store at room temperature. Product is shipped at ambient temperature. |

## Introduction

The Thermo Scientific B-PER Bacterial Protein Extraction Reagent (in phosphate buffer) was developed for extraction of soluble 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 and for inclusion body purification. This reagent contains a proprietary, mild, nonionic detergent in 50mM sodium phosphate (pH 7.5), which eliminates the need for mechanical disruption. The novel composition 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 and chelating agents may be added to the reagent.

## Important Product Information

- B-PER Reagent extracts proteins from recently prepared cells and frozen cells. Extraction is typically more effective with frozen cells. B-PER Reagent also extracts recombinant proteins from insect cells infected by baculovirus. The amount of reagent required depends on the confluence of the infected cells.
- If desired, add protease inhibitors (Product No. 78425 or 78430), salts, chelating agents, reducing agents and DNase I Solution (2,500U/mL) (Product No. 90083) directly to the reagent.
- Bacteria often over-express recombinant proteins and form inclusion bodies, which are insoluble aggregates of misfolded protein. Perform a mini-scale extraction to determine solubility of the specific recombinant protein before performing a large-scale protein extraction and purification. Centrifugation separates inclusion bodies from soluble proteins; however, Lysozyme (Product No. 89833) is required for purification of inclusion bodies. Lysozyme significantly improves inclusion body purity by digesting the cell debris.
- 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.
- Whole cell lysates prepared with the B-PER Reagent are compatible with the Thermo Scientific Pierce BCA Protein Assay (Product No. 23225); they also are compatible with the Thermo Scientific Coomassie Plus – The Better Bradford Assay Kit (Product No. 23236) when diluted 50% or treated with Thermo Scientific Compat-Able Protein Assay Reagent Set (Product No. 23215).
- The B-PER Reagent (in phosphate buffer) is supplied in a phosphate buffer system, therefore use phosphate-based buffers for subsequent protein purification.

## Example Procedures for Protein Extraction from Bacteria

**Note:** If sample becomes viscous after cell lysis, either add more B-PER Reagent or use DNase I Solution (2,500U/mL) (Product No. 90083) to degrade the nucleic acids.

### Mini-Scale Protein Extraction (1.5mL bacterial culture, A<sub>600</sub> = 1.5-3.0)

1. Pellet bacterial cells by centrifugation at 5000 × g for 10 minutes.
2. Resuspend cells in 300μL of B-PER Reagent by vigorously vortexing or pipetting up and down until the suspension is homogeneous. Vortex sample for 1 minute.

3. Centrifuge tube at  $15,000 \times g$  for 5 minutes to separate soluble proteins from the insoluble proteins.
4. Transfer supernatant (soluble fraction) to a new tube. Suspend pellet (insoluble fraction) in 300 $\mu$ L of B-PER Reagent. Use 10 $\mu$ L of the soluble and insoluble fractions for SDS-PAGE or Western blot to determine protein solubility. If purification of inclusion bodies is desired, proceed to the next step.
5. Just before use, prepare lysozyme in B-PER Reagent at 10mg/mL. Add 6 $\mu$ L of the lysozyme solution to the suspended pellet for a final concentration of 200 $\mu$ g/mL. Vortex tube for 1 minute.
6. Add 1mL of 1:10 diluted B-PER Reagent to the suspension and vortex for 1 minute.
7. Centrifuge tube at  $15,000 \times g$  for 10 minutes. Resuspend pellet in 1mL of 1:10 diluted B-PER Reagent and vortex for 1 minute. Repeat this step two more times. Proceed to the next step without resuspension.
8. Resuspend the inclusion body pellet in 300 $\mu$ L of ultrapure water or buffer. Use 10-20 $\mu$ L of sample for SDS-PAGE to determine purity.

**Midi-Scale Protein Extraction (40mL bacterial culture,  $A_{600} = 1.5-3.0$ )**

1. Pellet bacterial cells by centrifugation at  $5000 \times g$  for 10 minutes.
2. Add 5mL of B-PER Reagent to the pellet and vortex or pipetting up and down until the cell suspension is homogeneous. Gently shake the suspension for 10 minutes.
3. Separate soluble proteins from the insoluble proteins by centrifugation at  $15,000 \times g$  for 15 minutes. Transfer supernatant (i.e., soluble proteins) to a new tube. If purification of inclusion bodies is desired, proceed to the next step.  
**Note:** Typically >90% of the soluble proteins is recovered by this extraction. An additional extraction may increase yield.
4. To purify inclusion bodies, add 5mL of B-PER Reagent to the pellet and vortex or pipette up and down to resuspend.
5. Just before use, prepare lysozyme in B-PER Reagent at 10mg/mL. Add 100 $\mu$ L of the lysozyme solution to the suspension for a final concentration of 200 $\mu$ g/mL. Mix well and incubate at RT for 5 minutes.
6. Add 15mL of 1:10 diluted B-PER Reagent to the suspension and mix by vortexing.
7. Centrifuge tube at  $15,000 \times g$  for 15 minutes. Resuspend pellet in 20mL of 1:10 diluted B-PER Reagent. Repeat this step two more times. Proceed to the next step without resuspension.
8. Dissolve the purified inclusion bodies in denaturing agents and proceed to further refolding or purification procedures.

**Maxi-Scale Bacterial Protein Extraction (250mL bacterial culture,  $A_{600} = 1.5-3.0$ )**

**Note:** When using a larger volume of bacterial cultures, increase reagent volume accordingly.

1. Pellet bacterial cells by centrifugation at  $5000 \times g$  for 10 minutes.
2. Add 10-20mL of B-PER Reagent to the pellet and vortex or pipette up and down until the suspension is homogeneous. Gently shake the suspension for 10 minutes.
3. Centrifuge tube at  $15,000 \times g$  for 15 minutes. Transfer supernatant (i.e., soluble proteins) to a new tube. If purification of inclusion bodies is desired, proceed to the next step.  
**Note:** Typically >90% of the soluble proteins is recovered from this extraction. An additional extraction may increase yield.
4. To purify inclusion bodies, add 10-20mL of B-PER Reagent to the pellet and vortex or pipette up and down to resuspend.
5. Just before use, prepare lysozyme in B-PER Reagent at 10mg/mL. Add 200-400 $\mu$ L of the lysozyme solution to the suspension for a final concentration of 200 $\mu$ g/mL. Mix well and incubate at room temperature for 5 minutes.
6. Add 100mL of 1:10 diluted B-PER Reagent to the tube and vortex.
7. Centrifuge tube at  $15,000 \times g$  for 15 minutes. Resuspend pellet in 100mL 1:10 diluted B-PER™ Reagent. Repeat this step two more times. Proceed to the next step without resuspension.
8. Dissolve the purified inclusion bodies in denaturing agents and proceed to further refolding or purification procedures.

## Example Procedures for Protein Extraction from Insect Cells

### Example Method I (Monolayer Culture)

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

### Example Method II

1. Grow and infect insect cells according to standard protocols.
2. Collect cells by low-speed centrifugation and decant liquid.
3. Add 10mL of B-PER Reagent for each gram of wet cell pellet.
4. Resuspend pellet and shake suspension for 10 minutes.
5. Separate soluble proteins from the insoluble proteins (and cell debris) by centrifugation at  $15,000 \times g$  for 15 minutes.

## Related Products

|                   |   |
|-------------------|---|
| <b>78425</b>      | <b>Halt™ Protease Inhibitor Cocktail Kit</b>  |
| <b>78430</b>      | <b>Halt Protease Inhibitor Cocktail, EDTA-Free</b>  |
| <b>89833</b>      | <b>Lysozyme</b>   |
| <b>90083</b>      | <b>DNase I Solution (2,500U/mL)</b>   |
| <b>78115</b>      | <b>Inclusion Body Solubilization Reagent, 100mL</b>   |
| <b>XP04200BOX</b> | <b>Novex™ Tris-Glycine protein gels</b> (see <a href="http://thermofisher.com/proteingels">thermofisher.com/proteingels</a> for a complete listing) |
| <b>NW04120BOX</b> | <b>Bolt™ Bis-Tris Plus protein gels</b> (see <a href="http://thermofisher.com/proteingels">thermofisher.com/proteingels</a> for a complete listing) |
| <b>24615</b>      | <b>Imperial™ Protein Stain, 1L, coomassie R-250 stain</b>   |
| <b>LC6060</b>     | <b>SimplyBlue™ SafeStain</b>  |
| <b>24580</b>      | <b>Pierce™ Reversible Protein Stain Kit for Nitrocellulose Membranes</b>  |
| <b>26619</b>      | <b>PageRuler™ Plus Prestained Protein Ladder</b>  |
| <b>23227</b>      | <b>Pierce BCA Protein Assay Kit</b>   |
| <b>23236</b>      | <b>Pierce Coomassie Plus (Bradford) Assay Kit</b>   |
| <b>23208</b>      | <b>Pierce Bovine Serum Albumin (BSA) Pre-Diluted Set, 7 × 3.5mL of dilutions in the range of 125-2000µg/mL</b>                                      |

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