

B-PER Complete Bacterial Protein Extraction Reagent

89821 89822

2524.0

Number	Description
89821	B-PER Complete Bacterial Protein Extraction Reagent , 250mL, sufficient for lysis of 50g of bacterial biomass
89822	B-PER Complete Bacterial Protein Extraction Reagent , 500mL, sufficient for lysis of 100g of bacterial biomass

Composition: Solution contains a proprietary, buffered nonionic detergent with Thermo Scientific™ Pierce™ Universal Nuclease and lysozyme

Storage: Upon receipt store at 4°C. Product is shipped on ice packs.

Introduction

The Thermo Scientific™ B-PER™ Complete Bacterial Protein Extraction Reagent is an all-in-one formulation of nonionic detergent and enzymes that enables mild extraction of proteins from both Gram-negative and Gram-positive bacteria without the need for mechanical disruption. B-PER Complete Reagent includes Pierce Universal Nuclease and lysozyme to improve cell lysis and reduce viscosity and may be used for soluble protein extraction and inclusion body purification from bacterial cell lysates. Depending on the particular application, additional components such as protease inhibitors, salts, reducing agents and chelating agents may be added to the reagent. B-PER Complete Reagent is compatible with downstream applications such as affinity chromatography (e.g., immobilized metal affinity chromatography, glutathione chromatography), SDS-PAGE and protein quantification (e.g., Thermo Scientific™ BCA Protein Assay, Pierce™ 660nm Protein Assay).

Important Product Information

- The B-PER Complete Reagent extracts proteins from recently prepared and frozen cells. Extraction is more effective with frozen cells. The amount of reagent required depends on the amount of cell paste.
- For lysis of recently prepared Gram-negative cell paste, addition of 1mM EDTA is required for efficient lysis. Frozen Gram-negative cell pastes do not require addition of EDTA for lysis. No additional reagents are required for lysis of recently prepared or frozen Gram-positive bacteria.
- If desired, add Thermo Scientific™ EDTA-free Protease Inhibitors (Product No. 78425, 78437 or 78439), salts and reducing agents directly to the reagent. Do not exceed 1mM EDTA final concentration, because this may interfere with enzyme function and immobilized metal affinity chromatography.
- Some over-expressed proteins can be insoluble, misfolded or expressed in inclusion bodies. The best way to obtain soluble protein is to adjust the expression conditions; however, if this is not possible, the Thermo Scientific™ Inclusion Body Solubilization Reagent (Product No. 78115) can be used.
- B-PER Complete Reagent effectively extracts soluble proteins from several common bacterial host strains including Gram-negative and Gram-positive strains without the need for additional enzymes. If lysis is inefficient for a particular bacterial strain, freeze cells before extraction and/or increase lysis incubation time.

Procedure for Extracting Protein from Bacteria

1. Pellet bacterial cells by centrifugation at $5000 \times g$ for 10 minutes. Weigh biomass and proceed to Step 2 or freeze biomass at -20°C to -80°C .

2. Warm required amount of B-PER Complete Reagent (5mL reagent/g of biomass) to room temperature.

Optional: Add salts, reducing agents, chelators or protease inhibitors to B-PER Complete Reagent.

Note: Add 1mM EDTA final concentration to B-PER Complete Reagent (i.e., $2\mu\text{L}$ of 0.5M EDTA per mL of B-PER Complete Reagent) for lysis of recently prepared Gram-negative cells (e.g., *E. coli* BL21 strain).

3. Add 5mL of B-PER Complete Reagent per gram of cell pellet. Pipette the suspension up and down until it is homogeneous.

4. Incubate 15 minutes at room temperature with gentle rocking.

5. Centrifuge lysate at $16,000 \times g$ for 20 minutes to separate soluble proteins from the insoluble proteins.

Note: If a large percentage of over-expressed protein remains in the pellet, the protein of interest may be expressed in inclusion bodies. Use the Inclusion Body Solubilization Reagent (Product No. 78115) or alter the expression conditions to minimize inclusion body formation.

Troubleshooting

Problem	Possible Cause	Solution
Protein of interest is not solubilized	Protein of interest was expressed in inclusion bodies	Adjust expression conditions or use an inclusion body solubilization reagent
Low protein yield	Insufficient lysis of bacteria	Increase incubation time up to 1 hour
		Increase ratio of lysis buffer to cell pellet up to 10mL/g of biomass
		To improve lysis, use biomass that has undergone at least one freeze/thaw cycle
Lysate is viscous	Additional added reagents interfered with universal nuclease activity	Avoid using metal chelators at a concentration greater than 1mM
	B-PER Complete Reagent was incorrectly stored	Store solution at 4°C . Do not freeze or keep solution at temperatures greater than 4°C for extended periods of time

Additional Information Available on Our Website

- Protein Methods – Overview of Cell Lysis and Protein Extraction
- Tech Tip #43: Protein stability and storage
- Tech Tip #6: Extinction coefficients guide

Related Thermo Scientific Products

90084	B-PER Bacterial Protein Extraction Reagent, 250mL
78248	B-PER Bacterial Protein Extraction Reagent, 500mL
88701	Pierce Universal Nuclease for Cell Lysis, 25kU
88702	Pierce Universal Nuclease for Cell Lysis, 100kU
90082	Lysozyme, 0.5mL supplied at 50mg/mL
89965	HisPur™ Cobalt Resin, 100mL
88222	HisPur Ni-NTA Resin, 100mL
16100	Pierce Glutathione Agarose, 10mL
78115	Inclusion Body Purification Reagent, 100mL
87730	Slide-A-Lyzer™ G2 Dialysis Cassettes, 10K MWCO, 3mL, 10/pkg
87731	Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 15mL, 8/pkg
88665	Pierce Protease Inhibitor Mini Tablets, 30 tablets/vial
88666	Pierce Protease Inhibitor Mini Tablets, EDTA-Free, 30 tablets/vial
88265	Pierce Protease Inhibitor Tablets, 20 tablets/vial
88266	Pierce Protease Inhibitor Tablets, EDTA-Free, 20 tablets/vial
87786	Halt™ Protease Inhibitor Cocktail (100X), 1mL
87785	Halt Protease Inhibitor Cocktail, EDTA-Free (100X), 1mL
22660	Pierce 660nm Protein Assay Reagent, 750mL
23227	BCA Protein Assay Kit
24594	GelCode™ Blue Safe Protein Stain, 1L
24590	GelCode Blue Stain Reagent, 500mL, (coomassie G-250)
24615	Imperial™ Protein Stain, 1L, (coomassie R-250)

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