Bacterial PE LB™

Bacterial Protein Extraction Lysis Buffer

(Cat. # 786-176, 786-177, 786-185, 786-186, 786-187, 786-188)

INTRODUCTION

Bacterial PE LB™ has been developed for the extraction of soluble proteins and inclusion bodies from bacterial cells. It is a proprietary improvement on the lysozyme based lysis, which allows extraction of soluble proteins and concurrent removal of nucleic acids (DNA & RNA) released during cell lysis. The Bacterial PE LB™ lysis eliminates viscosity build-up, allowing effective clarification with lower centrifugal force. This kit is provided with an optional protocol for the formation of spheroplast and removal lytic enzyme (Lysozyme) prior to lysis and extraction of the bacterial proteins. Bacterial PE LB™ is based on organic buffering agents and utilizes a mild non-ionic detergent and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. Depending on the application, additional agents such as reducing agents, chelating agent, and protease inhibitors cocktail may be added into Bacterial PE LB™. This reagent has been tested for use with several widely used bacteria including E. coli strains.

Bacterial PE LB™ eliminates the need for laborious mechanical lysis of bacterial cells and removal of DNA/RNA with nuclease treatments. The proprietary combination of this reagent provides a simple and versatile method of bacterial protein extraction and isolation of inclusion bodies.

For bacterial lysis, the kits are suitable for extracting soluble proteins from approximately 30g wet cell pellets for every 100ml Bacterial PE LB™. When the kits are used for extracting soluble proteins from spheroplasts, they are suitable for approximately 9g wet cell pellets for every 25ml Bacterial Suspension Buffer supplied.

APPLICATIONS

Bacterial PE LB™ is suitable for preparation of spheroplast, lysis and extraction of proteins from bacterial cells and isolation of inclusion bodies.

COMPATIBILITY

Bacterial PE LB™ is compatible with most downstream applications including running various chromatography, gel electrophoresis applications, and protein folding procedures. Bacterial PE LB™ is also compatible for protein estimation with NI™ protein assay (Non-Interfering Protein Assay™, Cat# 786-005).

ITEM(S) SUPPLIED

	Bacterial PE LB™ Kit			Bacterial PE LB™ Buffer Only		
Cat. #	**786-176**	**786-187**	**786-188**	**786-177**	**786-185**	**786-186**
Bacterial PE LB™	100ml	250ml	500ml	500ml	100ml	250ml
Bacterial Suspension Buffer	25ml	60ml	125ml	-	-	-
PE LB™ Lysozyme (Cat# 786-042)	1ml	2 x 1ml	5ml	-	-	-

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival store the kit components at 4°C except store PE LB™ Lysozyme at -20°C. Stable for 1 year when stored and used as recommended.

ADDITIONAL ITEMS NEEDED

- Centrifuge
- Test tubes
- Incubator
- Additional volume of the Bacterial PE LB™ Buffer may be purchased separately for downstream applications such as chromatography, dialysis, etc.

PREPARATION BEFORE USE

Depending on applications, DTT and EDTA may be added. Prepare an appropriate volume of the Bacterial PE LB™ for use by adding DTT and EDTA both to a final concentration of 5mM. If the presence of a divalent metal ion is necessary for any application, do not add EDTA; instead, add an appropriate divalent salt to a final concentration of 5mM.

PE LB™ Lysozyme

The PE LB™ Lysozyme contains 40mg/ml Lysozyme (~80kU) supplemented with 800U/ml DNase and 24U/ml RNase. We recommend using the PE LB™ Lysozyme at a final concentration of 0.1-1mg/ml. Higher levels of lysozyme will not improve lysis efficiency and may have an inhibitory effect.

Protease Inhibition-

If the inhibition of protease activity is required, add a cocktail of protease inhibitors to prevent protease activities during extraction procedure. We recommend our Bacterial ProteaseArrest™ (Cat. # 786-330).

PROTOCOLS

A. Protein extraction with concurrent removal of nucleic acids

1. Pellet bacterial cells (bacterial culture, OD_{600} 1.5-3.0) by centrifugation at 5,000x g for 10 minutes.
2. Suspend the cell pellet in 5-10 volume of the Bacterial PE LB™ Buffer. For a 25µl cell pellet (50-75mg wet weight), use 125-250µl Bacterial PE LB™ Buffer).
3. Vortex for 1 minute or until the cell suspension is homogeneous. Incubate the suspension for 5 minutes in cold. Vortex again to suspend the cells.
4. Vortex the tube containing PE LB™ Lysozyme to mix the frozen suspension. Add an appropriate volume of PE LB™ Lysozyme to the cell suspension in Bacterial PE LB™ Buffer to give a final concentration of 0.1-1mg/ml. Gently mix the content.
   NOTE: *The starting concentration of PE LB™ Lysozyme is 40mg/ml. Additional amounts of PE LB™ Lysozyme may be purchased separately (Cat. # 786-042).*
5. Incubate the suspension at 37°C for 30-60 minutes.
   OPTIONAL:- *Lysis can be monitored by taking 25µl suspension and mixing with 1ml Bacterial PE LB™ Buffer and reading the optical density at OD 590nm.*
6. At the end of incubation period, vortex the content of the tube several times (30 seconds each) to complete the lysis. Lysis may be further assisted by pipetting the suspension up and down a few times with a narrow bore pipette tip or a 20-gauge syringe needle.
   NOTE: *Additional volume of the Bacterial PE LB™ Buffer may be purchased separately for downstream applications such as chromatography, dialysis, etc.*
7. ***Nucleic Acid Removal:*** During lysis, cellular DNA and RNA are cleaved, which reduces the viscosity of the lysate. Some DNA fragments may survive, however these would not interfere with downstream processing. For complete removal of nucleic acids, do not add EDTA in to the Bacterial PE LB™ Buffer. After lysis is complete EDTA may be added to a final concentration of 2.5mM.
8. Centrifuge the lysate at 20,000x g, 4°C for 30 minutes and collect the clear lysate.
9. Lysate is now ready for any application, including biological activity assays, electrophoresis, protein purification, or further analysis.
10. Titer Plate Applications: For high throughput titer plate applications the protocol can be modified by proportionately reducing the volumes.

B. Protein extraction with spheroplast formation

Suitable when *PE LB™* Lysozyme contamination is not acceptable.

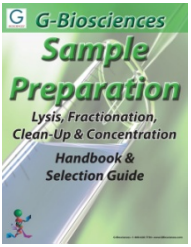
1. Pellet bacterial cells (bacterial culture, OD_{600} 1.5-3.0) by centrifugation at 5,000x g for 10 minutes. Suspend the cell pellet in 5-10 volume of the Bacterial Suspension Buffer (cell pellet size 25µl (50-75mg wet weight) use 125-250µl Bacterial Suspension Buffer).
2. Vortex for 1 minute or until the cell suspension is homogeneous. Incubate the suspension for 5 minutes at 4°C. Vortex again to suspend the cells.
3. Vortex the tube containing PE LB™ Lysozyme to mix the frozen suspension. Add an appropriate volume of PE LB™ Lysozyme to the cell suspension in Bacterial PE LB™ Buffer to give a final concentration of 0.1-1mg/ml. Gently mix the content.
   NOTE: *The starting concentration of PE LB™ Lysozyme is 40mg/ml. Additional amounts of PE LB™ Lysozyme may be purchased separately (Cat. # 786-042).*
4. Incubate the suspension at 37°C for 30-60minutes.
   OPTIONAL: *Lysis can be monitored by taking 25µl suspension and mixing with 1ml Bacterial PE LB™ Buffer and reading the optical density at OD 590nm.*
5. At the end of incubation, centrifuge the suspension at 5,000x g for 10 minutes. Remove and discard the supernatant carefully, leaving the spheroplast pellet in the tube.
   OPTIONAL*: Resuspend the spheroplast pellet in 5-10 volume of the Bacterial Suspension Buffer. Centrifuge again as above and discard the supernatant.*
6. **Lysis**: For lysis, suspend the spheroplast pellet in an appropriate volume of the Bacterial PE LB™ Buffer (2-3 times the volume of spheroplast pellet). Pipette the suspension up and down a few times. Vortex periodically and incubate on ice for 30 minutes. The lysis may be further facilitated by incubating the cells for 1-3 minutes at 37°C or a brief sonication step.
   NOTE: *The higher Bacterial PE LB™ Buffer to spheroplast pellet ratio the higher the level of cell lysis.*
7. Centrifuge the lysate at 20,000x g, 4°C for 30 minutes and collect the clear lysate.
   NOTE: *See section C below for the isolation of inclusion bodies.*
8. Lysate is now ready for any application, including biological activity assays, electrophoresis, protein purification, or further analysis.

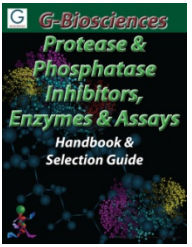
C. Isolation of Inclusion Bodies.

For inclusion bodies isolation, after the lysis step centrifuge the bacterial lysate at 30,000 x g for 30 minutes at 4°C. Collect the inclusion bodies pellet and wash twice with 10 fold diluted Bacterial PE LB™ Buffer (e.g., suspend in buffer and centrifuge to pellet the inclusion bodies). Collect the inclusion bodies for solubilization and re-folding.

RELATED PRODUCTS

Download our Sample Preparation and Protease & Phosphatase Inhibitors, Enzyme & Assays Handbooks.





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