Bacterial PE LB™ in Phosphate Buffer

Bacterial Protein Extraction Lysis Buffer

(Cat. #786-191)

INTRODUCTION

Bacterial PE LB™ in Phosphate buffer is a variation of Bacterial PE LB™ buffers. It is used preferably for extraction of soluble proteins and soluble recombinant proteins from bacterial cells. Bacterial PE LB™ in Phosphate buffer contains a mild non-ionic detergent, proprietary combination of various salts and agents in phosphate buffer. Bacterial PE LB™ in Phosphate buffer is mild lysis buffer with enhanced protein extraction and solubility.

Depending on the application, additional agents such as reducing agents, chelating agent, and protease inhibitors cocktail may be added into Bacterial PE LB™ in Phosphate buffer. This reagent has been tested for use with several widely used bacteria including E. coli strains.

Bacterial PE LB™ in Phosphate buffer eliminates the need for laborious mechanical lysis of bacterial cells. The proprietary combination of this reagent provides a simple and versatile method of bacterial protein extraction.

APPLICATIONS

Bacterial PE LB™ in Phosphate buffer is a variation of Bacterial PE LB™ buffers only which is used preferably for extraction of soluble proteins and soluble recombinant proteins from bacterial cells

COMPATIBILITY

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

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-191	Bacterial PE LB™ in Phosphate buffer	500 ml

STORAGE CONDITION

Bacterial PE LB™ in Phosphate buffer is shipped at ambient temperature. Upon arrival store it at 4°C.

ADDITIONAL ITEMS NEEDED

- PE LB™ Lysozyme (Cat# 786-042)
- Centrifuge
- Test tubes
- Incubator

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 (Cat. # 786-042)

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

Protein extraction with concurrent removal of nucleic acids

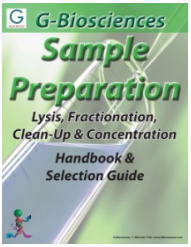
1. Pellet bacterial cells (bacterial culture, OD_{600} 1.5-3.0) by centrifugation at 5,000x g for 10 minutes.
2. Suspend the cell pellet in 5-10 volume of the Bacterial PE LB™ in Phosphate buffer. For a 25µl cell pellet (50-75mg wet weight), use 125-250µl Bacterial PE LB™ in Phosphate buffer.
3. Vortex 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™ in Phosphate 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.
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

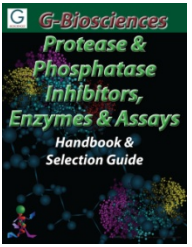
nucleic acids, do not add EDTA in to the Bacterial PE LB™ Buffer. After lysis is complete EDTA may be added to a final concentration of 2.5mM.

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.

RELATED PRODUCTS

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





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