G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Insect Cell-PE LB™

Insect Cell Protein Extraction & Lysis Buffer

(Cat. # 786-411)

INTRODUCTION

Insect Cell-PE LB™ has been developed for extraction of cytoplasmic soluble protein from insect cultured cells. The Insect Cell-PE LB™ is based on organic buffering agents, which 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 chelating agents, reducing agents and protease inhibitors may be added into Insect Cell-PE LB™ (see Related Products for protease inhibitor Protease Arrest™). Insect Cell-PE LB™ can be used for both suspension as well as adherent cells. The proprietary combination of this reagent provides a simple and versatile method for the extraction of protein from insect cells.

COMPATIBILITY

Insect Cell-PE LB™ is compatible with most applications, including enzyme assays, various chromatography procedures, electrophoresis, etc. Insect Cell-PE LB™ is also compatible for protein estimation with NI™ protein assay (Non-Interfering Protein Assay™). The protein extract prepared with Insect Cell-PE LB™ may be used for most enzyme assays including reporter gene assays (e.g. β-galactosidase, luciferase, chloramphenicol acetyltransferase), kinases (e.g., PKC, PKA, Tyrosine Kinase), and immunoassays (e.g., ELISA, Western blots, RIA).

ITEM(S) SUPPLIED (Cat. #786-411)

Description	Size
Insect Cell-PE LB™	250ml

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store at 4^0C. Stable for 1 year when stored and used as recommended.

ADDITIONAL ITEMS REQURIED

Centrifuge, test tubes, PBS, and Protease Inhibitor Cocktail.

PREPARATION BEFORE USE

Depending on applications, DTT and EDTA may be added. Prepare an appropriate volume of the Insect-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.

Protease Inhibition

If the inhibition of protease activity is required, add a cocktail of protease inhibitors to prevent protease activities during the extraction procedure. We recommend our ProteaseARREST™ protease inhibitor cocktail (Cat. # 786-108).

PROTOCOL FOR EXTRACTION FROM SUSPENSION-CULTURED INSECT CELLS

- *Centrifugal forces and handling techniques detailed in the procedure must be followed exactly to avoid partial lysis of insect cells, which may affect protein yield.*
- *Perform all lysis steps at 4˚C or on ice to decrease the rate of proteolysis.*

A. Harvest the Insect Cells

1. Determine the number of cells per milliliter of culture and then the total number of cells.
2. Harvest cells by centrifugation at 800 x g for 5 minutes at room temperature.
3. Decant the growth media and save the cell pellet.

B. Wash the Insect Cells

1. Add a volume of room temperature PBS to the pellet equal to the culture volume (e.g. if pellet is ~100µl in size add 100µl of PBS.) Gently resuspend cells by pipetting.
2. Centrifuge cells at 800 x g for 5 minutes at ambient temperature and decant the supernatant.
3. Repeat Steps 1-2.

C. Lyse the Insect cells

NOTE: *For best results, add protease inhibitors to the Insect Cell-PE LB™ immediately before use.*

1. Add 1 ml of Insect Cell-PE LB™ per 5 x 10^6-2 x 10^7 cells to the washed cell pellet.
2. Resuspend cells by pipetting up and down. Vortex cells for 5 seconds at medium speed.
3. Incubate cells on ice for 10 minutes.
4. Centrifuge cells at 15,000 x g for 15 minutes at 4˚C.
5. Carefully transfer the supernatant containing soluble proteins to a new tube. Avoid disrupting the pellet. Save the pellet, which contains insoluble protein and cellular debris, for further analysis.

PROTOCOL FOR EXTRACTION FROM MONOLAYER-CULTURED INSECT CELLS

A. Wash the Insect cells

1. Aspirate the media from the plate.
2. Gently add a volume of PBS to the plate that is equal to the culture volume. Be careful not to dislodge cells. Aspirate the PBS from the plate. Repeat this step.

B. Lyse the insect cells

NOTE: *For best results, add protease inhibitors to the Insect Cell-PE LB™ immediately before use.*

1. Add an appropriate volume of Insect Cell-PE LB™ according to the following table:

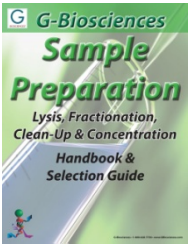
Plate Size/Surface Area	Volume of Insect Cell-PE LB™
100mm dish	500-1000µl
60mm dish	250-500µl
6-well plate	200-400µl per well
24-well plate	100-200µl per well
96-well plate	50-100µl per well
T-25 flask	500µl per flask
T-75 flask	1.5ml per flask

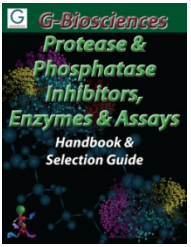
2. Incubate cells for 10 minutes at 4˚C. Incubate plates on a shaker platform with vigorous shaking. Tap flasks on the side or use a cell scraper. Cells should appear detached after 5-6 minutes.
3. Use a pipette to transfer the cells and debris to a new tube. Tilt the plate or flask to collect all material.
4. Centrifuge tube at 15,000 x g for 15 minutes at 4˚C.
5. Use a pipette to carefully transfer the supernatant containing soluble proteins to a new tube. Avoid disrupting the pellet. Save pellet containing insoluble protein and cellular debris for further analysis.

NOTE: *The cellular debris may contain some membrane bound protein, which may be further extracted with a variety of detergents. For more information on detergents, see Related Products: Proteomic Grade Detergents*.

RELATED PRODUCTS

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





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