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A Geno Technology, Inc. (USA) brand name

Mammalian Cell PE LB™

Mammalian Cell Protein Extraction & Lysis Buffer

(Cat. # 786-180)

INTRODUCTION

Mammalian Cell PE LB™ has been developed for extraction of total soluble proteins from mammalian cultured cells. The Mammalian 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 Mammalian Cell PE LB™. Mammalian Cell PE LB™ reagent has been tested for use with a wide variety of mammalian cells. Mammalian 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 proteins from mammalian cells.

COMPATIBILITY

Mammalian Cell PE LB™ is compatible with most applications, including enzyme assays, various chromatography procedures, electrophoresis, etc. Mammalian Cell PE LB™ is also compatible for protein estimation with NI™ protein assay (Non-Interfering Protein Assay™). The protein extract prepared with Mammalian 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. #786-180)

Description	Size
Mammalian Cell PE LB™	500ml

STORAGE CONDITIONS

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

ADDITIONAL ITEMS REQUIRED

Centrifuge, test tubes, and incubator

PREPARATION BEFORE USE

Depending on applications, DTT and EDTA may be added. Prepare an appropriate volume of the Mammalian 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 (see Related Products for protease inhibitor Protease *Arrest*™).

PROTOCOL

Lysis of Cell Suspension

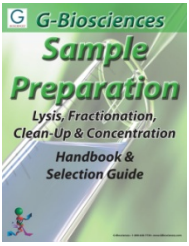
1. Pellet the cells by centrifugation at 3,000x g for 5 minutes. Remove and discard the supernatant. For adherent cells, scrape or detach cells from the culture plate, centrifuge and discard the supernatant.
2. Wash the cell pellet once with 5-10ml PBS. Pellet the cells again by centrifugation. Remove and discard the PBS wash.
3. Vortex and suspend the pellet in the remaining volume of PBS wash. Add Mammalian Cell PE LB™ and suspend the cell pellet. For each 10 ml of fully-grown suspension culture, add approximately 1ml Mammalian Cell PE LB™. Alternatively, add 1 ml Mammalian Cell PE LB™ lysis buffer for each 0.05 gram of wet cell pellet. For making even more concentrated cell extract, the volume of Mammalian Cell PE LB™ added to the pellet may be reduced. In such cases, one freeze and thaw cycle will ensure complete lysis of the cells.
4. Use a pipette to suspend the cells until you have a homogeneous suspension. Incubate the suspension on ice for 15-30 minutes. Periodically shake or briefly vortex the suspension.
   NOTE: *freeze and thaw step is not necessary for lysis, however, one or two freeze and thaw cycle is not detrimental to the cell extract, and often ensures complete lysis.*
5. Centrifuge the suspension at 20,000x g for 30 minutes in a refrigerated centrifuge. Collect the clear suspension for downstream processing and analysis.
   NOTE: *The cellular debris may contain some nuclear and membrane bound proteins, which may be further extracted with a variety of detergents (See Related Products - Proteomic Grade Detergents).*

Lysis of Adherent Mammalian Cells

1. Remove the culture medium from the adherent cells.
2. Wash the cells once with PBS. Remove the PBS wash.
3. Add an appropriate volume of the Mammalian Cell PE LB™ to cover the culture surface area:
    a. Add 50-100µl/well in 96 well plate
    b. Add 100-200µl/well in 24 well plate
    c. Add 200-400µl/well in 6 well plate
    d. Add 250-500µl/well in 60mm culture plate
    e. Add 500-1000µl/well in 100mm culture plate
4. Shake the culture plate gently for 10 minutes.
   NOTE: *If a more concentrated cell lysate is required, the volume of the Mammalian Cell PE LB™ added to the culture plate may be reduced as appropriate. Subject the culture plate or well to one cycle of freeze and thaw. Shake gently for 10 minutes.*
5. Lysate, including cellular debris may be used directly from the culture wells/plates. Alternatively, transfer the lysate to a centrifuge tube and centrifuge the lysate at 20,000 x g for 30 minutes. Collect the clear lysate for downstream processing and analysis.
   OPTIONAL: *Add NaCl to a final concentration of 0.1M NaCl (use a 2-4M NaCl solution). Addition of NaCl generally improves performance of many immuoassays.*
   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 Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook

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