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

A Geno Technology, Inc. (USA) brand name

Yeast PE LB™

Yeast Protein Extraction Lysis Buffer

(Cat. # 786-178, 786-179)

INTRODUCTION

Yeast PE LB™ is useful for extraction of soluble proteins from yeast cells. Yeast PE LB™ is a proprietary improvement on the Zymolyase® based spheroplast preparation and extraction of soluble proteins from yeast cells. This kit is provided with an optional protocol to make spheroplast and remove lytic enzyme Zymolyase®, prior to lysis and extraction of yeast proteins. Yeast PE LB™ is based on organic buffering agents that utilize a mild non-ionic detergent and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. A ready-to-use Zymolyase® preparation is also provided. Depending on application, additional agents such as reducing agents, chelating agents, and protease inhibitors may be added into Yeast- PE LB™ (see Related Products for protease inhibitor Protease Arrest™). The proprietary combination of this reagent provides a simple and versatile method of yeast protein extraction. Yeast PE LB™ eliminates the need for laborious glass bead lysis of yeast cells. The kit is good for 100 preps of 50µl yeast cell pellet.

APPLICATIONS

Preparation of yeast spheroplast and extraction of yeast proteins. This kit is suitable for processing approximately 10ml yeast cell pellet suspension, either single or multiple smaller preps.

COMPATIBILITY

Yeast PE LB™ is compatible with any downstream application including running various chromatography procedures and gel electrophoresis applications. Yeast PE LB™ is also compatible for protein estimation with NI™ protein assay.

ITEM(S) SUPPLIED

Description	Cat. # 786-178	Cat. # 786-179
Yeast PE LB™ Buffer	100ml	500ml
Yeast Suspension Buffer	15ml	-
Longlife™ Zymolyase® (1500U/ml)	2 x 0.5 ml	-

STORAGE CONDITIONS

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

ADDITIONAL ITEMS NEEDED

- Centrifuge
- Test tubes
- Incubator,
- DTT, EDTA and β-mercaptoethanol

Additional volume of the Yeast 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 Yeast 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 extraction procedure We recommend our ProteaseARREST™ Protease Inhibitor Cocktail (Cat. # 786-108).

PROTOCOL

1. Pellet Yeast cells (culture OD_{600} 1.5-2.0) by centrifugation at 5-10,000x g for 10 minutes. Suspend the cell pellet in an equal volume of the Yeast Suspension Buffer. Add 1µl of β-mercaptoethanol per 100µl Yeast suspension.
2. Vortex for 1 minute or until the cell suspension is homogeneous. Incubate the cell suspension for 5 minutes at 4°C. Vortex it again to suspend the cells.
3. Flick the vial containing Longlife™ Zymolyase® to mix the solution. Add 10µl Longlife™ Zymolyase® for each 100µl cell suspension. Gently mix the content.
4. Incubate the suspension at 37°C for 30-60 minutes. Lysis can be monitored by taking 25µl suspension, mixing with 1ml Yeast PE LB™ Buffer and reading optical density at 800nm.
5. At the end of incubation, centrifuge the suspension at 10,000x g for 5 minutes. Remove and discard the supernatant carefully, leaving the spheroplast pellet in the tube.

   ***Optional Washing**: Add 10 volumes (~600µl) of the Yeast Suspension Buffer to the spheroplast pellet. Re-suspend the spheroplast by gently tapping the tube. Centrifuge again as above and discard the supernatant.*

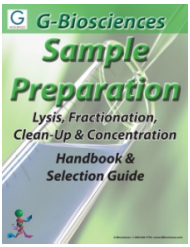
Spheroplast Lysis

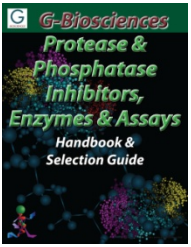
1. Suspend the yeast spheroplast pellet in 3 volumes of the Yeast PE LB™ Buffer. Pipette the suspension up and down a few times. Vortex periodically and incubate on ice for 30 minutes. Incubating the cells for 3 minutes at 37°C or a brief sonication step may further facilitate the lysis. Sonication is necessary for shearing genomic DNA.
   NOTE: *For better lysis, increase the Yeast PE LB™ to spheroplast ratio, because higher the ration better would be the lysis.*
2. Centrifuge at 20,000x g for 30 minutes at 4°C. Collect clear lysate. The lysate is now ready for purification of protein, other applications, or further analysis.

NOTE- *Additional volume of Yeast PE LB™ can be purchased separately for downstream applications e.g. chromatography and dialysis, etc.*

RELATED PRODUCTS

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





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