



Yeast-PE LB™

Yeast Protein Extraction Lysis Buffer

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.

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.

ITEMS INCLUDED:

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

STORAGE CONDITION

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.

PROTOCOL

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 (see our Related Products for protease inhibition - Protease Arrest™).

1. Pellet Yeast cells (culture OD₆₀₀ 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 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- Add 5-10 volume of the Yeast Suspension Buffer to the spheroplast pellet. Resuspend the spheroplast by gently tapping the tube. Centrifuge again as above and discard the supernatant.

6. Lysis: Suspend the yeast pellet (now spheroplast) in an appropriate volume of the Yeast-PE LB[™] Buffer (2-3 times the volume of cell pellet). Pipet the suspension up and down a few times. Vortex periodically and incubate on ice for 30 minutes. Incubating the cells for 1-3 minutes at 37°C or a brief sonication step may further facilitate the lysis. Sonication is necessary for shearing genomic DNA. Please note, the higher Yeast-PE LB[™] Buffer to yeast pellet ratio, the better will be cell lysis.
7. 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.

Zymolyase[®] is a registered trademark of Kirin Brewery Co. Ltd.

RELATED PRODUCTS

1. **Protease Arrest**[™] (Cat # 786-108): A cocktail of protease inhibitors for use during protein extraction and purification. Protease Arrest[™] inhibits a broad spectrum of serine, cysteine and metalloprotease as well as calpains.
2. **IBS**[™] (Cat # 786-183): Inclusion bodies solubilization buffer. For solubilization of isolated inclusion bodies, prior to re-folding protein procedures.
3. **Foldase**[™] (Cat# 786-185): A kit for optimization of protein folding procedure includes a selection of Foldase, i.e. agents to facilitate protein folding.
4. **Strategy**[™] (Cat# 786-184): A kit for optimization of purification of novel proteins includes a selection of agents, tools and simple to follow protocols.
5. **NI-Protein Assay**[™] (Cat#786-005): A protein assay that is free from interference of common laboratory agents, including reducing agents, detergents, dyes, EDTA, etc.

For more information visit our web site— www.GBiosciences.com or contact us.

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