

Yeast Protein Extraction Buffer Kit

Introduction

Yeast Protein Extraction Buffer Kit is useful for extraction of soluble proteins from yeast cells. Yeast Protein Extraction Buffer Kit is a proprietary improvement on the Zymolyase™ based spheroplast preparation and extraction of soluble proteins from yeast cells. This kit is provided with a protocol to make spheroplast and remove lytic enzyme Zymolyase, prior to lysis and extraction of yeast proteins. Yeast Protein Extraction Buffer is based on organic buffering agents, which utilize mild non-ionic detergents, 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 Protein Extraction Buffer Kit (see Related Products for protease inhibition – Protease Inhibitor Mix). The proprietary combination of this reagent provides a simple and versatile method of yeast protein extraction. Yeast Protein Extraction Buffer Kit 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 10 ml yeast cell pellet, either single or multiple smaller preps.

Compatibility

Yeast Protein Extraction Buffer Kit is compatible with any downstream application including enzyme assays, running various chromatography procedures, and gel electrophoresis applications.

Items included

Item	Size
Yeast Protein Extraction Buffer	100 ml
Yeast Suspension Buffer	15 ml
Longlife™ Zymolyase® (1500 U/ml)	2 × 0.5 ml

Storage condition

Shipped at ambient temperature. Upon arrival, store Longlife Zymolyase below -18°C and other kit components at 4–8°C. Stable for 1 year when stored and used as recommended.

Additional items

Centrifuge, test tubes, incubator, DTT, EDTA and β-mercaptoethanol.

Instructions for use

Yeast Protein Extraction Buffer Kit is intended for research use only.

Preparation Before Use

Depending on applications, DTT and EDTA may be added. Prepare an appropriate volume of the Yeast Protein Extraction Buffer for use by adding DTT and EDTA both to a final concentration of 5 mM. 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 5 mM.

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 Inhibitor Mix).

- 1 Pellet Yeast cells (culture OD600 1.5–2.0) by centrifugation at 3000 × g for 10 minutes.
Suspend the cell pellet in an equal volume of the Yeast Suspension Buffer.
Add 1 μl of β-mercaptoethanol per 100 μl 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 10 μl Longlife Zymolyase for each 100 μl cell suspension. Gently mix the content.
- 4 Incubate the suspension at 37°C for 30–60 minutes. Lysis can be monitored by taking 25 μl suspension, mixing with 1 ml Yeast Protein Extraction Buffer and reading optical density at 800 nm.
- 5 At the end of incubation, centrifuge the suspension at 10,000 × 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 Protein Extraction 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.

Note: The higher Yeast Protein Extraction Buffer to yeast pellet ratio, the better will be cell lysis.
- 7 Centrifuge at 20,000 × g for 30 minutes at 4°C. Collect clear lysate. The lysate is now ready for purification of protein, other applications, or further analysis.



Order information

Re-ordering Information for Yeast Protein Extraction Buffer Kit:

Product	Quantity	Code No.
Yeast Protein Extraction Buffer Kit	1	28-9440-45

Related products

Product	Quantity	Code No.
Nuclease Mix	0.5 ml	80-6501-42
Protease Inhibitor Mix	1 ml	80-6501-23
SDS-PAGE Clean-Up	Kit	80-6484-70
His SpinTrap™	50 × columns	28-4013-53
His MultiTrap™ HP	4 × 96-well plates	28-4009-89
His MultiTrap FF	4 × 96-well plates	28-4009-90
HisTrap™ FF crude	5 × 1 ml	11-0004-58
GST SpinTrap Purification Module	50 × columns, buffer kit	27-4570-03
GST MultiTrap FF	4 × 96-well plate	28-4055-01
GST MultiTrap 4B	4 × 96-well plate	28-4055-00
GSTrap™ 4B	5 × 1 ml	28-4017-45
PD-10 Desalting Columns	30 × columns	17-0851-01
VivaSpin™ ultracentrifugation devices	multiple	

For more information on these and other related products, visit our web site at www.gelifesciences.com or contact our technical dept.

For contact information for your local office, please visit, www.gelifesciences.com/contact

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