Protocol

TD-P Revision 3.0

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Yeast Cell Lysis Buffer

GoldBio Cat# GB-178 & GB-179

Introduction

The Yeast Lysis Buffer is useful for extraction of soluble proteins from yeast cells. It 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 spheroplasts and remove lytic enzyme Zymolyase®, prior to lysis and extraction of yeast proteins. Yeast Lysis Buffer 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 in GB-178.

Depending on application, additional agents such as reducing agents, chelating agents, and protease inhibitors may be added into Yeast Lysis Buffer (see Related Products for protease inhibitor *ProBlock Gold™*). The proprietary combination of this reagent provides a simple and versatile method of yeast protein extraction. Yeast Lysis Buffer eliminates the need for laborious glass bead lysis of yeast cells.

Applications

Preparation of yeast spheroplasts and extraction of yeast proteins: This kit is suitable for processing approximately 10 ml yeast cell pellet suspension, either single or multiple smaller preps. Yeast Lysis Buffer is compatible with any downstream application including running various chromatography procedures and gel electrophoresis applications.

Materials

Yeast Lysis Buffer plus Zymolyase (GoldBio Catalog # GB-178)

- Yeast Lysis Buffer
- Yeast Suspension Buffer
- Zymolyase® (1500U/ml)

Yeast Lysis Buffer (GoldBio Catalog # GB-179)

- Yeast Lysis Buffer

Additional materials

- Centrifuge
- Test tubes
- Incubator

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- Additional volume of the Yeast Lysis Buffer may be purchased separately for downstream applications such as chromatography, dialysis, etc.

Storage/Handling

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

Preparation Before Use

Depending on applications, DTT (GoldBio Catalog # DTT10) and EDTA (GoldBio Catalog # E-210) may be added. Prepare an appropriate volume of the Yeast Cell Lysis Buffer 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.

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 cocktails).

Method

Spheroplast Formation

1. Pellet yeast cells (bacterial culture, OD600 1.5-2.0) by centrifugation at 200-500 x g for 5-10 minutes. Suspend the cell pellet in an equal volume of Yeast Suspension Buffer. Add 1 µl of ß-mercaptoethanol per 100 µl yeast suspension.

2. Gently pipet up and down until the cell suspension is homogeneous. Incubate the suspension for 5 minutes at 4°C. Gently pipet again to suspend the cells.

3. Flick the vial containing Zymolyase® to mix the solution. Add 10 µl Zymolyase® for each 100 µl cell suspension in Yeast Lysis Buffer. Gently mix the content.

4. Incubate the suspension at 37°C for 30-60 minutes.

   Note: (OPTIONAL). Lysis can be monitored by taking 25 µl of suspension and mixing with 1 ml Yeast Lysis Buffer and reading the optical density at OD 800nm.

5. At the end of incubation, centrifuge the suspension at 10,000 x g for 5 minutes. Remove and discard the supernatant carefully, leaving the spheroplast pellet in the tube.

   Note: (OPTIONAL). Add 5-10 volumes 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.

Spheroplast Lysis

1. For lysis, suspend the spheroplast pellet in an appropriate volume of the Yeast Lysis Buffer (2-3 times the volume of spheroplast pellet). Pipet the suspension up and down a few times. Vortex periodically and incubate on ice for 30 minutes. The lysis may be further facilitated by incubating the cells for 1-3 minutes at 37°C or a brief sonication step. Sonication is necessary for shearing genomic DNA. Please note, the higher Yeast Lysis Buffer to spheroplast pellet ratio the better the cell lysis.

2. Centrifuge the lysed cells at 20,000 x g, for 30 minutes at 4°C and collect the clear lysate.

> **Note: Additional volume of Yeast Lysis Buffer can be purchased separately for downstream applications, e.g. chromatography and dialysis, etc.**

Lysate is now ready for any application, including biological activity assays, electrophoresis, protein purification, or further analysis.

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

Associated Products

- ProBlock™ Gold Yeast/Fungi (Cat # GB-333)**:** A cocktail of protease inhibition for use during protein extraction and purification from yeast or fungi. ProBlock Gold™ inhibits a broad spectrum of fungal serine, cysteine and metalloproteases as well as aspartic proteases.
- ProBlock™ Gold (Cat # GB-108)**:** A cocktail of protease inhibition for use during protein extraction and purification. ProBlock Gold™ inhibits a broad spectrum of serine, cysteine and metalloproteases as well as calpains.
- ProBlock™ Gold 2D (Cat # GB-109)**:** A cocktail of protease inhibition for use during protein sample preparation for IEF/2D studies. ProBlock Gold™ inhibits a broad spectrum of serine, cysteine and metalloproteases as well as aspartic proteases and aminopeptidases.