**Yeast Cell Lysis Buffer: GB-178 and GB-179**

**INTRODUCTION**
Yeast Lysis Buffer is useful for extraction of soluble proteins from yeast cells. Yeast Lysis Buffer 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 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 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 Lysis Buffer is compatible with any downstream application including running various chromatography procedures and gel electrophoresis applications.

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<th>ITEMS INCLUDED</th>
<th>Cat# GB-178</th>
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<tbody>
<tr>
<td>Yeast Lysis Buffer</td>
<td>100 ml</td>
<td>500 ml</td>
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<tr>
<td>Yeast Suspension Buffer</td>
<td>15 ml</td>
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<tr>
<td>Zymolyase®</td>
<td>2 x 0.5 ml</td>
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**STORAGE CONDITION**
Shipped at ambient temperature. Upon arrival store the kit components at 4°C except 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 Lysis 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 Lysis Buffer 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 – ProBlock Gold).

1. Pellet yeast cells (culture OD$_{600}$ 1.5-2.0) by centrifugation at 5-10000x g for 10 minutes. Suspend the cell pellet in an equal volume of Yeast Suspension Buffer. Add 1 µl of β-mercaptoethanol per 100 µl yeast suspension.
2. Cortex 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 Zymolyase® to mix the solution. Add 10 µl 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 1ml Yeast Lysis 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.
   a. **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 Lysis Buffer (2-3 times the volume of the 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 Lysis 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.
   a. **NOTE:** Additional volume of Yeast Lysis Buffer 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**
ProBlock Gold (Cat # GB-108): A cocktail of protease inhibitors for use during protein extraction and purification. ProBlock Gold™ inhibits a broad spectrum of serine, cysteine and metalloprotease as well as calpains.