BIO 101 Protocol

Yeast Cell Lysis Preparation Kit

2015-200	**10 preps**
2015-400	**25 preps**
2015-600	**100 preps**

- **For Use with the G NOME® DNA Isolation Kit and the RNaid® PLUS Kit**
- **Suitable for Genomic and Plasmid DNA Isolation**

Shipping & Storage:

Store all components at room temperature. Once **Spheroplasting Enzyme Mixx** is made store at -70°C.

"Check with BIO 101... We have you time in mind!"

BIO 101

Revision #: *2015-999-6A01P*

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Yeast Cell Lysis Protocol

Kit Components

2015-200 (10 preps)

Name	Volume	Catalog #
Yeast Suspension Buffer	10 ml	2015-201
Yeast Enzyme Enhancer	6 ml	2015-202
Spheroplast Indicating Solution	400 µl	2015-203
Spheroplast Control Solution	400 µl	2015-204
Yeast Enzyme Salts	120 µl	2015-205
Spheroplasting Enzyme Solution	800 µl	2015-206
Spheroplasting Enzyme Mixx	5.7 mg	2015-207

2015-400 (25 preps)

Name	Volume	Catalog #
Yeast Suspension Buffer	25 ml	2015-401
Yeast Enzyme Enhancer	14 ml	2015-402
Spheroplast Indicating Solution	1 ml	2015-403
Spheroplast Control Solution	1 ml	2015-404
Yeast Enzyme Salts	300 µl	2015-405
Spheroplasting Enzyme Solution	2 ml	2015-406
Spheroplasting Enzyme Mixx	14.25 mg	2015-407

2015-600 (100 preps)

Name	Volume	Catalog #
Yeast Suspension Buffer	100 ml	2015-601
Yeast Enzyme Enhancer	56 ml	2015-602
Spheroplast Indicating Solution	4 ml	2015-603
Spheroplast Control Solution	4 ml	2015-604
Yeast Enzyme Salts	1.2 ml	2015-605
Spheroplasting Enzyme Solution	4 x 2 ml	2015-606
Spheroplasting Enzyme Mixx	4 x 14.25 mg	2015-607

Introduction

The **Yeast Cell Lysis Preparation** Kit contains the necessary reagents for removal of the yeast cell wall in preparation for efficient lysis with the **G NOME**› DNA isolation kit (Catalog #'s: 2010-200, 2010-400, 2010-600). The rapid spheroplasting method employed in this protocol is suitable for genomic and plasmid DNA isolation. Transformation and tissue culture with these spheroplasts should not be performed following this method (for this protocol see **Yeast Spheroplast Transformation** Kit catalog #2210-200).

Procedure

1. Start with 100 ml of yeast grown to a density of 2 x 10^7 cells/ml.

2. Pellet at 600 xg for 5 minutes.

3. Discard supernatant and resuspend pellet in 1 ml of **Yeast Suspension Buffer**. Transfer to a microcentrifuge tube and centrifuge for 10 seconds.

4. Discard supernatant and resuspend in 500µl of **Yeast Enzyme Enhancer**.

5. Add 10µl **Yeast Enzyme Salts** and 80µl **Spheroplasting Enzyme Mixx.**

Before first use combine* Spheroplasting Enzyme Solution *with* Spheroplasting Enzyme Mixx. *Mix at room temperature for 5 minutes, aliquot into 80µl fractions (the solution often appears 'grainy' at this point). Quick freeze in liquid nitrogen. Store at -70°C until ready to use, only thaw once.

6. Incubate at 37°C until spheroplast formation is complete (usually 12-30 minutes).

How to Monitor Yeast Cell Lysis to Indicate Spheroplast Formation

Start monitoring spheroplast formation after 12 minutes of incubation. Allowing the reaction to proceed after spheroplasting is complete is not beneficial to subsequent procedures.

Place 20µl of **Spheroplast Indicating Solution** at one end of a glass slide and 20µl of **Spheroplast Control Solution** at the other end. After the cells have incubated for 12 minutes mix 2µl of yeast cells with each solution, cover with cover slips, and observe under the microscope. Spheroplasting is sufficient when less than 5% of the control number of cells remain intact in the visual field with the indicating solution relative to the control solution.

At this point cells are easily lysed using the general G NOME protocol.

Notes

Notes

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General Information

BIO 101 is a pioneer in developing kits for molecular biology research. We introduced the **GENECLEAN®** Kits in 1986 and have since been manufacturing products to bring convenience into your research. Our goal is to make your life easier by simplifying the complexities of lab work.

Technical Support and Ordering Information

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