

INSTRUCTIONS



Yeast Protein Kit™

Highlights

- ♦ Convenient method to lyse yeast cell for protein and PCR analysis.
- ♦ The kit can be used for any fungus species that are susceptible to yeast lytic enzyme digestion. It is optimized for *S. cerevisiae* and *C. albicans*.

Table of Contents

General Information.....	1
Package Contents	
Ordering Information	
General Description.....	2
Protocols.....	2
PCR Analysis	
Western Blot Analysis	



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GENERAL INFORMATION

Kit Contents*:

Product Y-Lysis Buffer	Storage Conditions Room Temperature
Zymolyase, (Resuspend the lyophilized enzyme by adding 200 ul of Storage Buffer to make 5 units/ul)	Shipped at room temperature. Store at -20°C after arrival
Instruction Sheet	

Reagents provided in this kit are designed for 200 protein analysis and are stable for 1 year.

Ordering Information:

Products	Cat No	Size
Yeast Protein Kit™ Reagents provided are for 200 protein analysis.	Y1002	1 kit
For Individual Sale:		
Y-Lysis Buffer	Y1002-1-6	6 ml
Zymolyase	E1004	1000 Units(lyophilized) Storage Buffer 500 ul

™ The Yeast Protein Kit, and Y-Lysis Buffer are trademarks of Zymo Research. Zymolyase™ is a trademark of the Kirin Brewery Co., Ltd. For Research use only. Always wear protective gloves and eye protection. These reagents are intended for use by trained professionals. Further precautions should be taken according to your own company's regulations.

GENERAL DESCRIPTION

The Yeast Protein Kit is a simple and convenient method to lyse yeast cells for protein analysis, such as Western Blot Analysis, and PCR amplification of plasmid or genomic DNA. The kit essentially generates spheroplasts of yeast cells for subsequent analysis. It is optimized for *S. cerevisiae* and *C. albicans*. The kit can also be used for any fungus species that is susceptible to yeast lytic enzyme digestion.

Before Starting: Add 200 ul of the supplied **Storage Buffer** to the lyophilized **Zymolyase™**. Mix to dissolve the enzyme completely, spin briefly in a micro-centrifuge. Store the reconstituted **Zymolyase™** at -20°C.

Western Blot Analysis

This method produces spheroplasts of yeast cells. Fresh and log-phase cells are more susceptible to **Zymolyase**. Try to use fresh cells whenever possible.

1. Spin down $1 \times 10^{5-6}$ cells, and remove the supernatant.
Use 200-500 μ l yeast liquid culture in most situation. Remove supernatant as much as possible.
2. Add 25 μ l of Y-Lysis Buffer and 1 μ l of **Zymolyase** to the sample.
For multiple sample analysis, add 40 μ l of **Zymolyase** to 1 ml of **Y-Lysis Buffer**. Use 25 μ l of this mixture for each sample.
3. Incubate at 37°C for 30-60 minutes.
- 4a. Add 25 μ l of 2X SDS-PAGE sample buffer to the digested yeast cell suspension and the sample can be used directly for SDS_PAGE analysis. If the experiment requires remove of the Y-Lysis Buffer, use procedure in step 4b below.
- 4b. Centrifuge the cell at 500 g for 5 minutes and remove the supernatant. The pellet can be directly resuspended in SDS-PAGE sample buffer for gel analysis.

PCR Analysis

Two protocols are provided. In the protocol I, simply add 0.5-1 μ l of Zymolyase directly to each PCR reaction (20-100 μ l reaction) plus yeast cells. Protocol II makes yeast spheroplast first, and then the spheroplast is used as a template for PCR analysis. Both methods work.

PCR Protocol I

1. Remove a small amount of yeast cells, ~0.1-0.5 μ l from a colony or pellet, and add it directly to the PCR reaction.
2. Add 0.5 μ l of **Zymolyase** to each 20-100 μ l of PCR reaction. Proceed to PCR directly.
If the PCR is set-up at room temperature, proceed directly to PCR reaction. If the PCR is set-up on ice, incubate sample at 37°C for 5 minutes before starting the PCR reaction.

PCR Protocol II

This method is suitable for both liquid cultures and colonies.

1. Pellet approximately $1 \times 10^{6-7}$ yeast cells, and remove the supernatant.
For colonies, pick a small amount of yeast cells, ~0.5-2 μ l volume, using a pipette tip and dispense into the lysis solution in Step 2 below.
2. Add 20 μ l of **Y-Lysis Buffer** and 1 μ l of **Zymolyase** to the sample.
For multiple sample analysis, add 40 μ l of **Zymolyase** to 1 ml of **Y-Lysis Buffer**. Use 25 μ l of this mixture for each sample.
3. Incubate at 37°C for 5-10 minutes.

The cell suspension is ready for PCR reactions. Use 2-4 μ l of this digested cell suspension for each PCR reaction. The remaining portion can be frozen for future use.