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*.

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The Beauty of Science is to Make Things Simple

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GENERAL INFORMATION

Kit Contents*:

Product	Storage Conditions
Y-Lysis Buffer	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 -20ºC 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 protien analysis.	**Y1002**	1 kit
For Individual Sale:		
Y-Lysis Buffer	Y1002-1-6	6 ml
Zymolyase	E1004	1000 Units(lyophilized) Storage Buffer 500 ul

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 $1x10^{5-6}$ cells, and remove the supernatant.
   Use 200-500 ul yeast liquid culture in most situation. Remove supernatant as much as possible.

2. Add 25 μl of Y-Lysis Buffer and 1 μl of **Zymolyase** to the sample.
   For multiple sample analysis, add 40 μl of **Zymolyase** to 1 ml of **Y-Lysis Buffer**. Use 25 μl of this mixture for each sample.

3. Incubate at 37°C for 30-60 minutes.

4a. Add 25 ul 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 μl of Zymolyase directly to each PCR reaction (20-100 μl 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 μl from a colony or pellet, and add it directly to the PCR reaction.

2. Add 0.5 μl of **Zymolyase** to each 20-100 μl 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 approximatle $1x10^{6-7}$ yeast cells, and remove the supernatant.
   For colonies, pick a small amount of yeast cells, ~0.5-2 μl volume, using a pipette tip and dispense into the lysis solution in Step 2 below.

2. Add 20 μl of **Y-Lysis Buffer** and 1 μl of **Zymolyase** to the sample.
   For multiple sample analysis, add 40 μl of **Zymolyase** to 1 ml of **Y-Lysis Buffer**. Use 25 μl 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 ul of this digested cell suspension for each PCR reaction. The remaining portion can be frozen for future use.