

# Zymolyase<sup>®</sup> (from *Arthrobacter luteus*)

Zymolyase<sup>®</sup>, produced by a submerged culture of *Arthrobacter luteus*<sup>(1)</sup>, has strong lytic activity against living yeast cell walls<sup>(2),(3)</sup> to produce protoplast or spheroplast of various strains of yeast cells. An essential enzyme for the lytic activity of Zymolyase<sup>®</sup> is  $\beta$ -1,3-glucan laminaripentaohydrolase. It hydrolyzes linear glucose polymers with  $\beta$ -1,3-linkages and releases specifically laminaripentaose as the main and minimum product unit<sup>(4), (5), (10), (11)</sup>.

There are two preparations of Zymolyase<sup>®</sup>, Zymolyase<sup>®</sup>-20T and Zymolyase<sup>®</sup>-100T, having lytic activity of 20,000 units/g and 100,000 units/g respectively. Zymolyase<sup>®</sup>-20T is ammonium sulfate precipitate while Zymolyase<sup>®</sup>-100T is a further purified preparation by affinity chromatography<sup>(9)</sup>. Lytic activity varies depending on yeast strain, growth stage of yeast, or cultural conditions<sup>(6-8)</sup>. Further informations related to Zymolyase<sup>®</sup> can be obtained in the reference section below<sup>(12-16)</sup>.



## Product Information

Product Name	Zymolyase <sup>®</sup> -20T	Zymolyase <sup>®</sup> -100T	
Form	Lyophilized Powder		
Purification	Ammonium Sulfate Precipitation	Affinity Chromatography	
Activity	20,000 units/g	100,000 units/g	
Essential enzyme	$\beta$ -1,3-glucan laminaripentaohydrolase		
Other activities contained <sup>(1)</sup>	$\beta$ -1,3-glucanase	approx. $1.5 \times 10^6$ units/g	approx. $1.0 \times 10^7$ units/g
	protease	approx. $1.0 \times 10^4$ units/g	approx. $1.7 \times 10^4$ units/g
	mannanase	approx. $1.0 \times 10^6$ units/g	approx. $6.0 \times 10^4$ units/g
Contaminants	Amylase, Xylanase, Phosphatase	Trace amount	Non detectable
Optimum pH and Temp.	pH7.5, 35°C (for lysis of viable yeast cells) pH6.5, 45°C (for hydrolysis of yeast glucan)		
Stability	2°C	No loss of activity was found after storage for 1 year	
Heat stability	30°C	70% of the lytic activity is lost after storage for 3 months	90% of the lytic activity is lost after storage for 3 months
	60°C	Lytic activity is lost on incubation for 5 minutes	
Specificity (Lytic Spectrum)	<i>Ashbya</i> , <i>Candida</i> , <i>Debaryomyces</i> , <i>Eremothecium</i> , <i>Endomyces</i> , <i>Hansenula</i> , <i>Hanseniaspora</i> , <i>Kloeckera</i> , <i>kluyveromyces</i> , <i>Lipomyces</i> , <i>Metschnikowia</i> , <i>Pichia</i> , <i>Pullularia</i> , <i>Torulopsis</i> , <i>Saccharomyces</i> , <i>Saccharomycopsis</i> , <i>Saccharomycodes</i> , <i>Schwanniomyces</i> , etc.		

(\*1) See reference, Kitamura, K., Kaneko, T., Yamamoto, Y., *J. Gen. Appl. Microbiol.*, **18**, 57 (1972) as to the definition of each enzyme units.

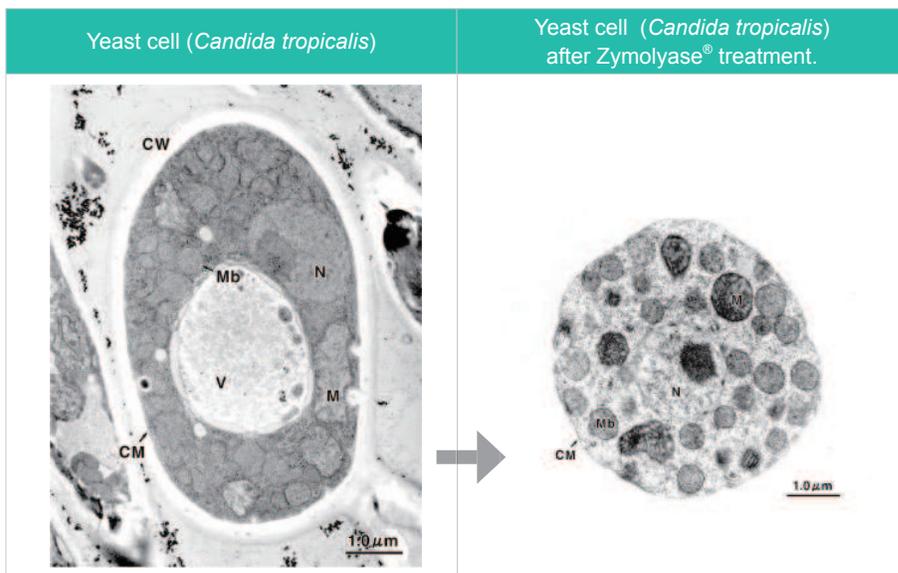
## Unit Definition

One unit of lytic activity is defined as that amount which indicates 30% of decrease in absorbance at 800 nm ( $A_{800}$ ) of the reaction mixture under the following condition.

[Reaction Mixture]	
Enzyme solution	: 1 ml (0.05-0.1 mg/ml for Zymolyase <sup>®</sup> -20T) (0.012-0.024 mg/ml for Zymolyase <sup>®</sup> -100T)
Brewer's yeast cell suspension	: 3 ml (2 mg/ml)
1/15M Phosphate buffer	: 5 ml (pH7.5)
Distilled water	: 1 ml

After incubation for 2 hours at 25°C with gentle shaking,  $A_{800}$  of the mixture is determined. When 60% of  $A_{800}$  decrease, equivalent to 2 units, is observed in the reaction system, the brewer's yeast cells are completely lysed, namely 1 unit of Zymolyase<sup>®</sup> lyses 3 mg dry weight of brewer's yeast.

## Electron microscopical photo of yeast cell



CW: Cell Wall  
CM: Cell Membrane  
M: Mitochondria

Mb: Microbody  
N: Nucleus  
V: Vacuole

(Data courtesy of Masako Osumi, Emeritus Professor at Nippon Women's University)

### Precaution on use

1. Avoid using nitrocellulose filters and use of material other than nitrocellulose when sterilizing. Zymolyase® may be adsorbed on nitrocellulose membranes.
2. Zymolyase® -100T may not be completely dissolved in buffers. Use Zymolyase® as suspension.
3. When sterilized, Zymolyase® is used in a concentration higher than 0.05%, prepare 2% Zymolyase® solution in buffers containing 5% glucose, filter the suspension and dilute the solution with the appropriate buffer.

### Reference

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### Ordering Information

Product Name	Storage	Product No.	PKG Size
Zymolyase®-20T	R	07663-91	1 g
Zymolyase®-100T	R	07665-55	500 mg

Zymolyase® is a registered trademark of Kirin Holdings Company, Limited.

For research use only, not intended for diagnostic or drug use.

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