

Zymolyase™ (from Arthrobacter luteus)

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

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



Product Information

Product Name		Zymolyase™-20T	Zymolyase™-100T
Form		Lyophilized Powder	
Purification		Ammonium Sulfate Precipitation	Affinity Chromatography
Activity		20,000 units/g	100,000 units/g
Essential enzyme		β-1,3-glucan laminaripentaohydrolase	
Other activities contained(*1)	β-1,3-glucanase	approx. 1.5 x 10 <sup>6</sup> units/g	approx. 1.0 x 10 <sup>7</sup> units/g
	protease	approx. 1.0 x 10 <sup>4</sup> units/g	approx. 1.7 x 10 <sup>4</sup> units/g
	mannanase	approx. 1.0 x 10 <sup>6</sup> units/g	approx. 6.0 x 10 <sup>4</sup> 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)		Ashbya, Candida, Debaryomyces, Eremothecium, Endomyces, Hansenula, Hanseniaspora, Kloeckera, kluyveromyces, Lipomyces, Metschnikowia, Pichia, Pullularia, Torulopsis, Saccharomyces, Saccharomycopsis, Saccharomycodes, Schwanniomyces, 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.

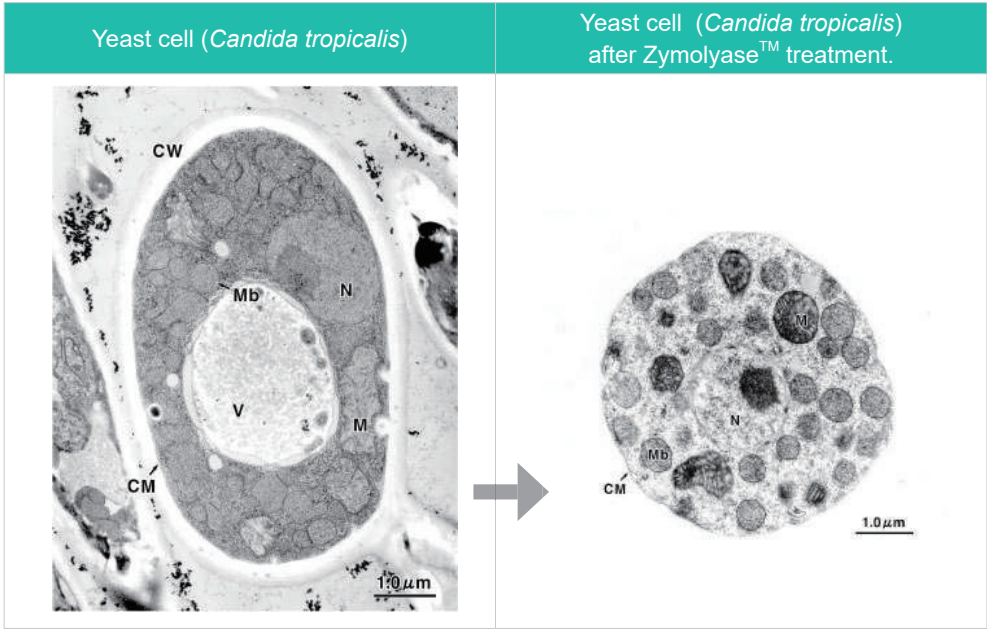
Unit Definition

One unit of lytic activity is defined as that amount which indicates 30% of decrease in absorbance at 800 nm (A800) of the reaction mixture under the following condition.

- [Reaction Mixture]
Enzyme solution : 1 ml (0.05-0.1 mg/ml for Zymolyase™-20T)
(0.012-0.024 mg/ml for Zymolyase™-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, A800 of the mixture is determined. When 60% of A800 decrease, equivalent to 2 units, is observed in the reaction system, the brewer's yeast cells are completely lysed, namely 1 unit of Zymolyase™ lyses 3 mg dry weight of brewer's yeast.

Electron microscopical photo of yeast cell



CW: Cell Wall Mb: Microbody  
CM: Cell Membrane N: Nucleus  
M: Mitochondria 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

1. Kaneko, T., Kitamura, K and Yamamoto, Y.: J. Gen. Appl. Microbiol., **15**, 317 (1969)  
2. Kitamura, K., Kaneko, T. and Yamamoto, Y.: Arch. Biochem. Biophys., **145**, 402 (1971)  
3. Kitamura, K., Kaneko, T. and Yamamoto, Y.: J. Gen. Appl. Microbiol., **18**, 57 (1972)  
4. Kitamura, K. and Yamamoto, Y.: Arch. Biochem. Biophys., **153**, 403 (1972)  
5. Kaneko, T., Kitamura, K. and Yamamoto, Y.: Agric. Biol. Chem., **37**, 2295 (1973)  
6. Kitamura, K., Kaneko, T. and Yamamoto, Y.: J. Gen Appl. Microbiol., **20**, 323 (1974)  
7. Kitamura, K. and Yamamoto, Y.: Agric. Biol. Chem., **45**, 1761 (1981)  
8. Katamura, K. and Tanabe, K.: Agric. Biol. Chem., **46**, 553 (1982)  
9. Katamura, K.: J. Ferment. Technol., **60**, 257 (1982)  
10. Kitamura, K.: Agric. Biol. Chem., **46**, 963 (1982)  
11. Kitamura, K.: Agric. Biol. Chem., **46**, 2093 (1982)  
12. Calza R. E., Schroeder A. L.: J. Gen. Microbiol., **129**, 413 (1983)  
13. Iizuka Masaru, Torii Yasuhiko, Yamamoto Takehiko: Agric. Biol. Chem., **47** (12), 2267 (1983)  
14. Shibata Nobuyuki, Kobayashi Hidemitsu, Tojo Menehiro, Suzuki Shigeo: Arch. Biochem. Biophys., **251**(2), 697 (1986)  
15. Iijima Y., Yanagi S. O.: Agric. Biol. Chem., **50** (7), 1855 (1986)  
16. Herrero Enrique, Sanz Pascual, Sentandreu Rafael: J. Gen. Microbiol., **133** (10), 2895 (1987)

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.

NACALAI TESQUE, INC.

Nijo Karasuma, Nakagyo-ku, Kyoto 604-0855 JAPAN  
TEL : +81-(0)75-251-1730  
FAX : +81-(0)75-251-1763  
Website : www.nacalai.com  
E-mail : info.intl@nacalai.com