

Product No. 07663

ZYMOLYASE[®]-20T (from *Arthrobacter luteus*)

Source: *Arthrobacter luteus*

Description: ZYMOLYASE[®]-20T, produced by a submerged culture of *Arthrobacter luteus*¹⁾, is a new enzyme preparation which lyses effectively cell walls of viable yeast cells^{2), 3)}. This preparation is a lyophilized powder prepared by salting out from culture fluid with ammonium sulfate.

An essential enzyme responsible for lysis of viable yeast cells in this preparation is β -1,3-glucan laminaripentaohydrolase. It hydrolyzes linear glucose polymers with β -1,3-linkages and releases specifically laminaripentaose as the main and minimum product unit^{4, 5, 10, 11)}.

The extent of lysis of yeast cells by ZYMOLYASE[®]-20T varies with yeast strain, growth stage of yeast, or cultural condition⁶⁻⁸⁾.

ZYMOLYASE[®]-20T shows 20,000 units/g of the lytic activity, defined after, toward brewer's yeast cells (*Saccharomyces cerevisiae*, resting stage) or toward yeast cells of *Saccharomyces cerevisiae* IFO 0565 cultured statically in malt extract medium (malt extract 2g, peptone 0.5g, water 100ml) at 20°C for 34hr.

Further purified preparation⁹⁾ is also available as ZYMOLYASE[®]-100T whose specific activity is 100,000units/g.

Further informations related to ZYMOLYASE[®] are obtained in the references cited below¹²⁻¹⁶⁾.

Specifications:

Activity	20,000units/g	
Contaminants	β -1, 3-glucanase	1.5 × 10 ⁶ units/g
	Protease	1.0 × 10 ⁴ units/g
	Mannanase	1.0 × 10 ⁶ units/g
	(See reference No.3 as to the definition of each enzyme units. Each activity varies more or less amount lots.)	
	Amylase, Xylanase, Phosphatase	Minute amounts
Essential Enzyme	β -1, 3-glucan laminaripentaohydrolase	
Appearance	Lyophilized powder	
Optimum pH and temperature	pH7.5, 35°C (for lysis of viable yeast cells) pH6.5, 45°C (for hydrolysis of yeast glucan)	
Stable pH	5-10	
Heat stability	The lytic activity is lost on incubation at 60°C for 5 minutes.	
Specificity (Lytic spectrum) ⁵⁾	<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.	
Activator	SH compound such as cysteine, 2-mercaptoethanol or dithiothreitol	

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

Assay for Enzyme Activity:

Method

[Reaction mixture]

Substrate and Buffer solution:	Brewer's yeast cell suspension (2mg dry weight/ml)	3mL
	M/15 Phosphate buffer, pH7.5	5mL
Enzyme solution:	0.05-0.1mg/mL solution	1mL
Distilled water		1mL
Total volume		10mL

[Procedure]

After incubation for 2 hours at 25°C with gentle shaking, A₈₀₀ of the mixture is determined. As a reference, 1ml of distilled water is used instead of enzyme solution.

Calculation

Percentage decrease in A₈₀₀ = (A₈₀₀ of reference – A₈₀₀ of reaction mixture) × 100/ initial A₈₀₀ of reference

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®-20T lyses 3mg dry weight of brewer's yeast.

Precautions on use: Use a sterilized filter except nitrocellulose when a sterilized enzyme solution is needed.

Storage: Stable for at least 1 year at 2°C. About 70% of the lytic activity is lost when stored at 30°C for 3 months.

References:

- 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) Kitamura K. and Tanabe, K.: *Agric. Biol. Chem*, **46**, 553(1982)
- 9) Kitamura, 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. and Schroeder A. L.: *J. Gen. Microbiol.*, **129**, 413(1983)
- 13) Iizuka, M., Torii, Y. and Yamamoto, T.: *Agric. Biol. Chem.*, **47**(12), 2767(1983)
- 14) Shibata, N., Kobayashi, H., Tojo, M. and Suzuki, S.: *Arch. Biochem. Biophys.*, **251**(2), 697(1986)
- 15) Iijima, Y. and Yanagi, S. O.: *Agric. Biol. Chem.*, **50**(7), 1855(1986)
- 16) Herrero, E., Sanz, P. and Sentandreu, R.: *J. Gen. Microbiol.*, **133**(10), 2895(1987)

Note: For *in vitro* research use only, not for diagnostic or therapeutic use. This product is not a medical device.

Central Laboratories for
Frontier Technology
Kirin Brewery CO., Ltd.