

CATALOG # 120491-1  
Package Size: 1 g (20KU/ mg)

Zymolyase® -20T  
(from *Arthrobacter luteus*)

Zymolyase® -20T, produced by a submerged culture of *Arthrobacter luteus*<sup>(1)</sup>, is a new enzyme preparation which lyses effectively cell walls of viable yeast cells<sup>(2, 3)</sup>. 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<sup>(4, 5, 10, 11)</sup>.

The extent of lysis of yeast cells by Zymolyase® -20T varies with yeast strain, growth stage of yeast, or cultural condition<sup>(6 - 8)</sup>.

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 uvarum* IFO 0565 cultured statically in malt extract medium (Malt extract 2 g, peptone 0.5 g, water 100 ml) at 20 °C for 34 hours.

Further purified preparation<sup>(9)</sup> is also available as Zymolyase® -100T whose specific activity is 100,000 units/g. Further information related to the Zymolyase® are obtained in the references cited below<sup>(12 - 16)</sup>.

**SPECIFICATIONS:**

<b>Appearance:</b>	Lyophilized powder	
<b>Activity:</b>	20,000 units/g	
<b>Essential enzyme:</b>	β-1, 3-glucan laminaripentaohydrolase	
<b>Other activities contained:</b>	β-1, 3-glucanase	ca. 1.5 x 10 <sup>6</sup> units/g
	protease	ca. 1.5 x 10 <sup>4</sup> units/g
	mannanase	ca. 1.5 x 10 <sup>6</sup> units/g
	(See reference No. 3 as to the definition of each enzyme unit. Each activity varies more or less among lots.)	
	DNase, RNase	not detected
<b>Optimum pH and temperature:</b>	pH 7.5, 35 °C (for lysis of viable yeast cells) pH 6.5, 45 °C (for hydrolysis of yeast glucan)	
<b>Stable pH:</b>	5-10	
<b>Heat stability:</b>	The lytic activity is lost on incubation at 60 °C for 5 minutes.	
<b>Specificity (lytic spectrum)<sup>(5)</sup>:</b>	Ashbya, Candida, Debaryomyces, Eremothecium, Endomyces, Hansenula, Hanseniaspora, Klöckera, Kluyveromyces, Lipomyces, Metschnikowia, Pichia, Pullularia, Torulopsis, Saccharomyces, Saccharomycopsis, Saccharomycodes, Schwanniomyces, etc.	
<b>Activators:</b>	SH compound such as cystein, 2-mercaptoethanol or dithiothreitol	

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## ASSAY FOR ENZYME ACTIVITY:

### 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.

### Method

#### Reaction mixture

Enzyme:	0.05-0.1 mg/ml solution	1 ml
Substrate:	Brewer's yeast cell suspension (2 mg dry weight/ml)	3 ml
Buffer:	M/15 Phosphate buffer, pH 7.5	5 ml
Distilled water:		1 ml
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Total Volume:		10 ml

### Procedure

After incubation for 2 hours at 25°C with gentle shaking,  $A_{800}$  of the mixture is determined. As a reference, 1 ml of distilled water is used instead of enzyme solution.

### Calculation

Percentage decrease in  $A_{800}$  = ( $A_{800}$  of reference -  $A_{800}$  of reaction mixture) x 100/initial  $A_{800}$  of reference 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®-20T lyses 3 mg 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 least 1 year at 2°C. About 70% of the lytic activity is lost when stored at 30°C for 3 months.

### REFERENCES:

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- 16) Herrero Enrique, Sanz Pascual. Sentandreu Rafael: J. Gen. Microbiol., 133 (10), 2895 (1987)

### NOTE:

This product is for laboratory use only - not for drug, household or other uses.

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