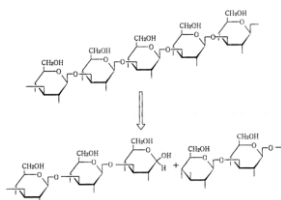


## Zymolyase® 20T

#GE013.0001 (1g)  
(FOR RESEARCH ONLY)



**Product:** Zymolyase® 20T (or Lyticase) from GRiSP is prepared from *Arthrobacter luteus*, and is supplied as an ammonium sulfate precipitate of a complex of enzymes. The strong lytic activity against living yeast cell walls is mainly due to the activity of  $\beta$ -1,3-glucan laminaripentaohydrolase. This enzyme hydrolyzes linear glucose polymers with  $\beta$ -1,3-linkages and releases specifically laminaripentaose as the main and minimum product unit, allowing for the production of protoplasts or spheroplasts of various yeast strains.



Lytic activity varies depending on yeast strain, growth stage of yeast, or cultural conditions. Under the conditions mentioned below, the lytic activity of Zymolyase® 20T is 20,000 U/g.

**Applications:** Preparation of spheroplasts or protoplasts from a variety of yeast strains.

**Content:** #GE013.0001 contains 1g of lyophilized Zymolyase® 20T.

<b>Properties:</b>	Activity	
	$\beta$ -1,3-glucan laminaripentaohydrolase:	20,000 U/g
	Other Activities	
	$\beta$ -glucanase:	$\sim 1.5 \times 10^6$ U/g
	Protease:	$\sim 1.0 \times 10^4$ U/g
	Mannanase:	$\sim 1.0 \times 10^6$ U/g
	Contaminants	
	Amylase:	trace amounts
	Xylanase:	trace amounts
	Optimum Conditions (pH, temp)	
	For lysis of viable yeast cells:	pH 7.5 – 35°C
	For hydrolysis of yeast glucan:	pH 6.5 – 45°C
	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>kluveromyces</i> , <i>Lipomyces</i> , <i>Metschnikowia</i> , <i>Pullularia</i> , <i>Saccharomyces</i> , <i>Saccharomycopsis</i> , <i>Schwanniomyces</i> , <i>Torulopsis</i> , <i>Saccharomycodes</i> , <i>Pichia</i> , etc.

\*One Unit (U) of lytic activity is defined as the amount that results in a 30% decrease of the absorbance at 800nm of a reaction mixture<sup>1)</sup> after incubation with gentle shaking at 25°C for 2 hours. A decrease of  $A_{800}$  with 60%, equivalent to 2U, corresponds to complete lysis. In other words, 1U of Zymolyase® lyses 3mg of dry weight *S. cerevisiae* in 2 hours under ideal conditions.

<sup>1)</sup> 10 ml reaction mixture is: 1 ml of distilled water, 3 ml of *Saccharomyces cerevisiae* (2 mg/ml), 5 ml 1/15 M phosphate buffer (pH 7.5), and 1 ml of enzyme solution.

**Storage:** Store the lyophilized powder at +4°C.

### Usage:

Add 0.1mg (2U) of Zymolyase® per 1ml of 1/15M Phosphate Buffer pH 7.5 and (optional) filter sterilize (see precautions).

*One liter of Phosphate Buffer 1/15 M can be prepared by mixing 800 ml of ultrapure water containing 19.1 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  with 200 ml of ultrapure water containing 1.814 g of  $\text{KH}_2\text{PO}_4$ . Enzyme solution can also be prepared in other phosphate buffers at pH 7.5 (e.g., 5 mM, 10mM or 50 mM Potassium phosphate buffer or 5 mM, 10mM or 50 mM Sodium phosphate buffer), with only slight loss of activity.*

The prepared solution is stable at +2°C to +8°C for 1 year (No loss of activity) or at +30°C (70% of lytic activity is lost after 3 months). At +60°C, all lytic activity is lost after 5 minutes.

### Precautions:

- Zymolyase® can be adsorbed onto nitrocellulose membranes. Avoid nitrocellulose filters when sterilizing.
- If Zymolyase® is to be used at higher concentrations than 0.1%, prepare a 2% Zymolyase® solution (20 mg/ml) in a buffer containing 5% glucose. Dilute the suspension with the appropriate buffer, filter sterilize, and store aliquots at 2-8°C.