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 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).

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).

Specifications

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


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®-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, 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® lyzes 3 mg dry weight of brewer’s yeast.
Electron microscopical photo of yeast cell

Yeast cell (*Candida tropicalis*)

Yeast cell (*Candida tropicalis*) after Zymolyase® treatment.

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


Ordering Information

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Storage</th>
<th>Product No.</th>
<th>PKG Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zymolyase®-20T</td>
<td>R</td>
<td>07663-91</td>
<td>1 g</td>
</tr>
<tr>
<td>Zymolyase®-100T</td>
<td>R</td>
<td>07665-55</td>
<td>500 mg</td>
</tr>
</tbody>
</table>

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

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