Duotech srl
Via Monte Spluga 31 - 20021 Baranzate (Milano)
tel 02.33106630 fax 02.33106640
www.duotech.it info@duotech.it

"Quo Fata Vocant"

ZYMOLYASE™

Zymolyase®, purified from culture fluid of *Arthrobacter luteus*, has strong lytic activity against living yeast cell walls to produce protoplast or spheroplast of various strains of yeast cells. Essential enzyme lytic activity of Zymolyase® is β-1, 3-glucan laminaripentaohydrolase, which hydolyzes glucose polymers linked by β-1, 3-bonds and produces laminaripentaose. Zymolyase® is reported to be a complex enzyme of Zymolyase A, β-1, 3-glucan laminaripentaohydrolase and Zymolyase B, alkaline protease, which may change the structure of the yeast cell wall to facilitate penetration of Zymolyase A. Zymolyase A alone was unable to lyse yeast cell walls. There are two preparations of Zymolyase®, Zymolyase® 20T and 100T, having lytic activity of 20,000 units/gram and 100,000 units/gram respectively. Zymolyase® 20T is ammonium sulphate precipitate while Zymolyase® 100T is a further purified preparation by affinity chromatography. Lytic activity varies depending on strains, fermentation conditions and growth phases of yeast substrate.

Form: Lyophilized powder

Purification:
Zymolyase® 20T: $(NH_4)_2SO_4$ precipitation
Zymolyase® 100T: Affinity Chromatography

Activity:
Zymolyase® 20T: 20,000 units/gram
Zymolyase® 100T: 100,000 units/gram

Essential enzyme: ß-1,3-glucan laminaripentaohydrolase

Other activities contained:

	Zymolyase®-20T	Zymolyase®-100T
ß-1,3-glucanase	ca. 1.5×10^6 units/g	ca. 1.0×10^7 units/g
protease	ca. 1.0×10^4 units/g	ca. 1.7×10^4 units/g
mannanase	ca. 1.0×10^6 units/g	ca. 6.0×10^4 units/g

Contaminants: Trace amounts of amylase, xylanase, phosphatase. No DNase, RNase detected

Optimum pH & temperature: pH 7.5, 35°C (for lysis of viable yeast cells)
pH 6.5, 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]: Ashbya, Candida, Debaryomyces, Eremothecium, Endomyces, Hansenula, Hanseniaspora, Kloeckera, Kluyveromyces, Lipomyces, Metschikowia, Pichia, Pullularia, Torulopsis, Saccharomyces, Saccharomycopsis, Saccharomycodes, Schwanniomyces, etc.

Activity: SH compound such as cystein, 2-mercaptoethanol of dithiothreitol

Stability: No loss of activity was found after storage for 1 year at 4°C

PROPERTIES OF ZYMOLYASE

Lytic Spectrum by Zymolyase®

1) **Susceptible strains in low concentration (0.2 units/ml)**
Ashbya, Endomyces, Kloeckera, Kluyveromyces, Pullularia, Saccharomyces

2) **Susceptible strains in high concentration (2.0 units/ml)**
Candida, Debaryomyces, Eremothecium, Hansenula, Hanseniaspora, Lipomyces, Metschikowia, Saccharomycopsis, Saccharomycodes, Schizosaccahromyces, Selenozyma, Trigonopsis,Wickerhamia

3) **Susceptibility depending on strains**
Bretanomyces, Cryptococcus, Nadsonia, Pichia, Rodosporidium, Schwanniomyces, Stephnoascus, Torulopsis

4) **No susceptible strains**
Bullera, Pityrosporum, Rhosotorula, Sporidiobolus, Sporobolomyces, Stetigmatomyces, Trichosporon

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.

Reaction mixture

Enzyme Solution:	0.05-0.1 mg/ml for Zymolyase® 20T 0.012-0.024 mg/ml for Zymolyase® 100T	1 ml
Substrate:	Brewer's yeast cell suspension (2 mg dry weight/ml)	3 ml
Buffer:	M/15 Phosphate buffer, pH 7.5	1 ml
Distilled water:		1 ml

Procedure

After incubation for 2 hours at 25°C with gentle shaking, A800 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 or Zymolyase® 100T lyses 3 mg dry weight of brewer's yeast.

PRECAUTIONS 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, especially 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.

APPLICATIONS:

- Protoplast/spheroplast preparation
- Yeast cell fusion
- Transformation of yeast cells
- Yeast genetics

STORAGE:

Stable for least 1 year at 2°C. When stored at 30°C for 3 months, about 70% of the lytic activity is lost in Zymolyase® 20T and 90% in Zymolyase® 100T.

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. Hen. 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,.: *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. Ben. 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)

Cat. Number	Description	Package Size
120491-1	Zymolyase® 20T	1 g (20KU/g)
120493-1	Zymolyase® 100T	500 mg (100KU/g)