Labiase

Cat#OZK-OZ30-EX

from *Streptomyces fulvissimus* TU-6.

Labiase, produced by a submerged culture of *Streptomyces fulvissimus* TU-6, is a new enzyme preparation that lyses effectively cell walls of numerous lactic acid bacterium. This preparation is used as a tool for studies of cell walls structure of lactic acid bacterium and the preparation of plasmid DNA, intracellular enzyme and protoplast from lactic acid bacterium and so on.

SPECIFICATIONS:

Appearance.....Lyophilized powder (containing lactose)
Package Size 500mg
Activity.....≧10 units/g (β-N-Acetyl-D-glucosaminidase)
Other activities contained Lysozyme≧2×10^4 units/g
Optimum pH and temperature.....See Fig. 1,2
pH and thermal stability .....See Fig. 1,2

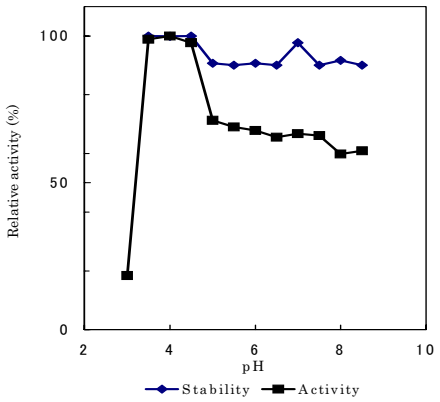

Fig.1 pH-stability and activity
Stability:18 hours treatment at 25℃
Activity:37℃

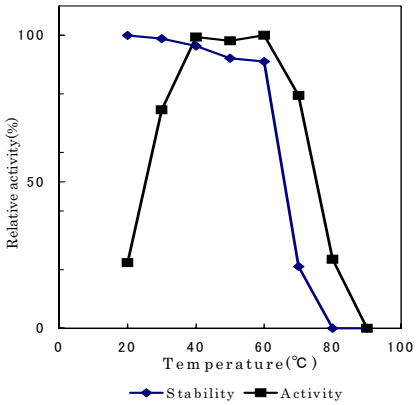

Fig.2 Thermal-stability and activity
Stability:10 minutes treatment
Activity:pH4.0

ASSAY FOR β-N-ACETYL-D-GLUCOSAMINIDASE ACTIVITY:

Unit Definition

One unit of enzyme catalyzes the release of 1 μ mole of *p*-nitrophenol from *p*-nitrophenyl N-acetyl-β-D-glucosaminide per minute at 37℃, pH4.0.

Method

Reaction mixture

Substrate.....5mM *p*-nitrophenyl N-acetyl-β-D-glucosaminide in 0.2M Citrate-phosphate buffer, pH 4.0		0.95ml
Enzyme.....suitably diluted enzyme		0.05ml
	Total volume	1.00ml

Procedure

After incubation for 10 minutes at 37℃, terminate by 2ml of 1M Na_2CO_3. Read A_{405}.

Calculation

$$\text{Enzyme unit} = \frac{A_{405} \times 3}{17.7 \times 10^3 \times 10} \times 10^3$$

The molar extinction coefficient of free *p*-nitrophenol under these conditions is 17,700.

ASSAY FOR LYTIC ACTIVITY TOWARD LACTIC ACID BACTERIUM

Method

Reaction mixture

Buffer.....50mM Citrate -sodium citrate buffer, pH 4.0	1ml
Substrate...Lactic acid bacterium suspension(A_{660}≒5.0)	1ml
Enzyme.....500 μ g-protein/ml solution	1ml
Distilled water	2ml
Total volume	5ml

Procedure

Before adding the enzyme solution preincubation is carried out for 5 minutes at 37℃.After incubation for 2 hours at 37℃ with gentle shaking, A_{660} of the mixture is determined. As a reference, 1 ml of distilled water is used instead of enzyme solution.

Calculation

$$\text{Percentage decrease in } A_{660} = \frac{(d0-dt)-(D0-Dt)}{d0} \times 100$$

d(0 or t):A_{660} of reaction mixture after 0 or 2 hours
D(0 or t):A_{660} of reference mixture after 0 or 2 hours

Result

See Table 1.

The extent of lysis of lactic acid bacterium cells by **Labiase** varies with lactic acid bacterium strain, growth stage of lactic acid bacterium, or cultural condition.

STORAGE:

Lyophilized preparation is stable for at least 1 year when stored at 4℃.

Table 1.Lysis of lactic acid bacterium cells by **Labiase**.

Strains	Lysis(%)
Leuconostoc mesenteroides IFO 3832	92.8
Leuconostoc lactis IFO 12455	99.6
Streptococcus faecalis IFO 3971	96.5
Lactobacillus acidophilus IFO 13951	97.9
Lactobacillus plantarum IAM 1041	96.8
Lactobacillus casei subsp. casei IFO 3533	59.4
Lactobacillus casei subsp. ramnosus IAM 1118	91.8
Lactobacillus brevis IFO 3345	95.6
Lactobacillus fermentum IFO 3071	70.4
Lactobacillus sp.(buchneri) IFO 3961	89.4
Lactobacillus fructivorans IFO 13954	96.9
Lactobacillus delbrueckii subsp. bulgaricus JCM 1002	97.7
Lactobacillus delbrueckii subsp. delbrueckii JCM 1012	92.0
Lactobacillus helveticus JCM 1120	98.7
Lactobacillus helveticus JCM 1005	84.5
Lactobacillus kefir JCM 5818	93.0
Lactobacillus sanfrancisco JCM 5818	82.1
Bifidobacterium bifidum JCM 1255	62.9
Streptococcus salivaris subsp.thermophilus IFO 13957	94.0
Streptococcus mutans IFO 5705	73.9

REFERENCES:

1) Ohbuchi, K., Hasegawa, K., Hamachi, M., Ozeki, K., and Kumagai, C.(2001) J. Biosci.Bioeng.91, 487.

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