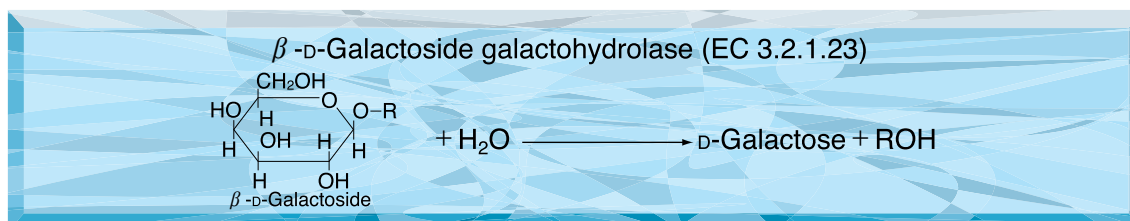


● TOYOBO ENZYMES ●

(Diagnostic Reagent Grade)

β -GALACTOSIDASE

from *Escherichia coli*



PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized.
Activity	: Grade II 500U/mg-solid or more
Contaminants	: α -galactosidase <math>< 1 \times 10^{-4}\%</math> α -glucosidase <math>< 1 \times 10^{-4}\%</math> β -glucosidase <math>< 2 \times 10^{-3}\%</math> α -mannosidase <math>< 1 \times 10^{-4}\%</math> β -mannosidase <math>< 1 \times 10^{-4}\%</math> proteinase <math>< 10\text{mAbs/mg-P}</math>
Stabilizer	: Mg ⁺⁺

PROPERTIES

Stability	: Stable at -20°C for at least 6 months	(Fig.1)
Molecular weight	: 540,000 ^{1,2)}	
Isoelectric point ³⁾	: 4.61	
Michaelis constants	: 3.0 × 10 ⁻⁴ M (o-Nitrophenyl- β -D-galactoside), 6.7 × 10 ⁻⁵ M (p-Nitrophenyl- β -D-galactoside), 2.3 × 10 ⁻⁴ M (Phenyl- β -D-galactoside), 2.5 × 10 ⁻³ M (Lactose)	
Structure ^{4~8)}	: The enzyme is composed of four identical subunits having a molecular weight of ca.135,000. The amino acid analysis indicates approximately 1,170 residues per subunit. $E_{1\text{cm}}^{280\text{nm}}(1\%) = 20.9$	
Inhibitors	: p-Chloromercuribenzoate, Iodoacetamide, heavy metal ions (Zn ⁺⁺ , Fe ⁺⁺⁺ , Cd ⁺⁺ , Cu ⁺⁺ , Pb ⁺⁺ , Ag ⁺ , Hg ⁺⁺), Ionic detergents (SDS, DAC, etc.)	
Optimum pH	: 7.0—7.5	(Fig.2)
Optimum temperature	: 50—55°C	(Fig.3)
pH Stability	: pH 6.5—8.5 (25°C, 20hr)	(Fig.4)
Thermal stability	: below 50°C (pH 7.3, 15min)	(Fig.5)
Substrate specificity	: The enzyme specifically hydrolyzes β -D-galactosyl linkage (Table 1).	
Effect of various chemicals	: (Table 2)	

APPLICATIONS

This enzyme is useful for structural investigation of carbohydrates, the determination of lactose (foodstuff analysis) and as an enzyme label for enzyme immunoassay.

ASSAY

Principle:

o-Nitrophenyl- β -D-galactopyranoside (ONPG) $\xrightarrow{\beta\text{-galactosidase}}$ o-Nitrophenol (ONP) + D-Galactose
The appearance of o-nitrophenol is measured at 410nm by spectrophotometry.

Unit definition:

One unit causes the formation of one micromole of ONP per minute under the conditions described below.

Method:

Reagents

- A. Phosphate buffer, pH 7.3 : 0.1M (Prepare by mixing 0.1M Na₂HPO₄ and 0.1M KH₂PO₄ to reach pH 7.3 at 37°C.)
- B. Mercaptoethanol solution : 3.36M [Dilute 4.0ml of 2-mercaptoethanol (14.2M) to 17ml with H₂O.]
(Should be prepared fresh)
- C. MgCl₂ solution : 30mM (Dissolve 610mg of MgCl₂ · 6H₂O in about 80ml of H₂O and, after adjusting the pH to 7.3 with 1.0 N NaOH, fill up to 100ml with H₂O.)
- D. ONPG solution : 34mM (205mg ONPG/20ml of Reagent A)(Stable for one week if stored at 0–5°C)
- E. Enzyme diluent : 50mM phosphate buffer, pH 7.3 contg. 1.0mM MgCl₂ and 0.1% BSA

Procedure

- Prepare the following reaction mixture in a cuvette (d = 1.0cm) and equilibrate at 37°C for about 5 minutes.

Concentration in assay mixture	
Phosphate buffer	92 mM
ONPG	2.3mM
Mercaptoethanol	0.11 M
MgCl ₂	1.0mM

2.5ml	0.1M Phosphate buffer, pH 7.3	(A)
0.1ml	Mercaptoethanol solution	(B)
0.1ml	MgCl ₂ solution	(C)
0.2ml	ONPG solution	(D)

- Add 0.1ml of the enzyme solution* and mix by gentle inversion.
- Record the increase in optical density at 410nm against water for 2 to 3 minutes in a spectrophotometer thermostated at 37°C, and calculate Δ OD per minute from the initial linear portion of the curve (Δ OD test).

At the same time, measure the blank rate (Δ OD blank) by using the same method as the test except that the enzyme diluent is added instead of the enzyme solution.

- * Dilute the enzyme preparation to 0.17–0.85U/ml with ice-cold enzyme diluent (E).

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta \text{OD}/\text{min} (\Delta \text{OD test} - \Delta \text{OD blank}) \times V_t \times df}{3.5 \times 1.0 \times V_s} = \Delta \text{OD}/\text{min} \times 8.57 \times df$$

V_t : Total volume (3.0ml)

V_s : Sample volume (0.1ml)

3.5 : Millimolar extinction coefficient of ONP under the assay condition (cm²/micromole)

1.0 : Light path length (cm)

df : Dilution factor

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Table 1. Substrate Specificity of β -Galactosidase

Substrate (2.3mM)	Relative activity	Vmax** (Relative value)	Substrate (2.3mM)	Relative activity	Vmax** (Relative value)
o-Nitrophenyl- β -D-galactopyranoside	100	100	p-Nitrophenyl- α -D-mannopyranoside	0	0
p-Nitrophenyl- β -D-galactopyranoside	14.7	13.4	p-Nitrophenyl- β -D-mannopyranoside	0	0
Phenyl- β -D-galactopyranoside*	1.1	1.3	p-Nitrophenyl- α -L-fucopyranoside	0	0
Lactose	2.1	3.9	p-Nitrophenyl- β -L-fucopyranoside	0	0
p-Nitrophenyl- α -D-galactopyranoside	0	0	p-Nitrophenyl- α -D-xylopyranoside	0	0
p-Nitrophenyl- α -D-glucopyranoside	0	0	p-Nitrophenyl- β -D-xylopyranoside	0	0
p-Nitrophenyl- β -D-glucopyranoside	0	0			

*Liberation of galactose was measured using galactose dehydrogenase as a coupling enzyme.

**Vmax was obtained from Lineweaver-Burk plots (Vmax with o-Nitrophenyl- β -D-galactopyranoside was 1,000 micromoles of hydrolyzed substrate per min per mg-protein).

Table 2. Effect of Various Chemicals on β -Galactosidase

[This enzyme dissolved in 50mM PIPES buffer, pH 7.0(10U/ml) was incubated with each chemical at 30°C for 30minutes. The residual activity was assayed according to the routine method described above.]

Chemical	Concn.(mM)	Residual activity	Chemical	Concn.(mM)	Residual activity
None	—	100%	MIA	2.0	86
Metal salt	2.0		NEM	2.0	95
MgCl ₂		99	IAA	2.0	1.4
CaCl ₂		102	Hydroxylamine	2.0	78
Ba(OAc) ₂		80	EDTA	5.0	103
FeCl ₃		59	o-Phenanthroline	2.0	99
CoCl ₂		83	α, α' -Dipyridyl	2.0	103
MnCl ₂		100	Borate	50	98
ZnSO ₄		6.2	NaF	2.0	99
Cd(OAc) ₂		4.7	NaN ₃	20	98
NiCl ₂		77	Triton X-100	0.1%	101
CuSO ₄		0.9	Brij 35	0.1%	103
Pb(OAc) ₂		1.3	Tween 20	0.1%	103
AgNO ₃		0	Span 20	0.1%	107
HgCl ₂		2.0	Na-cholate	0.1%	109
Mercaptoethanol	2.0	99	SDS	0.05%	75
Cysteine	2.0	102	DAC	0.05%	0
PCMB	2.0	0.3			

Ac, CH₃CO; PCMB, p-Chloromercuribenzoate; MIA, Monoiodoacetate; NEM, N-Ethylmaleimide; IAA, Iodoacetamide; EDTA, Ethylenediaminetetraacetate; SDS, Sodium dodecyl sulfate; DAC, Dimethylbenzyl-alkyl-ammonium chloride.

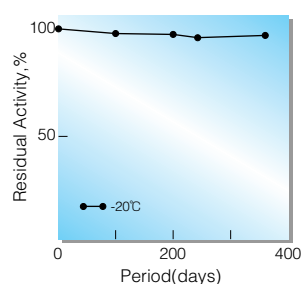


Fig.1. Stability (Powder form)
[kept under dry conditions]

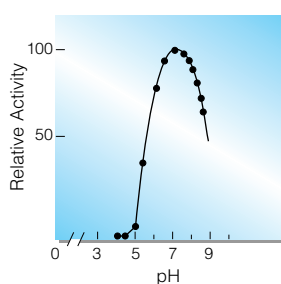


Fig.2. pH-Activity
[37°C, 15 min-reaction in Britton-Robinson buffer]

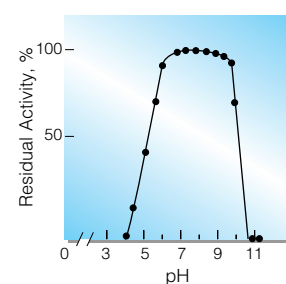


Fig.4. pH-Stability
[25°C, 20hr-treatment with Britton-Robinson buffer]

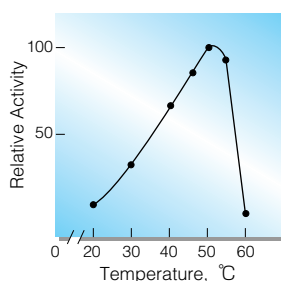


Fig.3. Temperature activity
[15min-reaction in 0.1M phosphate buffer, pH7.3]

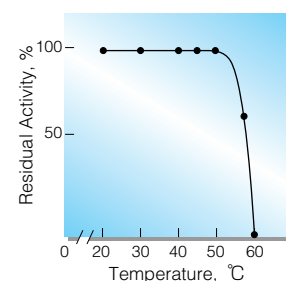


Fig.5. Thermal stability
[15min-treatment with 50mM phosphate buffer, pH7.3 contg. 1.0mM MgCl₂ enzyme concn.:80U/ml]

活性測定法 (Japanese)

1. 原理

o-Nitrophenyl- β -D-galactopyranoside (ONPG)

$\xrightarrow{\beta\text{-galactosidase}}$ o-Nitrophenol(ONP)+D-Galactose

o-Nitrophenolの生成量を410nmの吸光度の変化で測定する。

2. 定義

下記条件で1分間に1マイクロモルのo-Nitrophenolを生成する酵素量を1単位 (U)とする。

3. 試薬

- A. 0.1Mリン酸緩衝液, pH7.3 (37°C)(0.1M Na₂HPO₄溶液と0.1M KH₂PO₄溶液を混合して37°CでpHを7.3に調製する)
- B. 3.36Mメルカプトエタノール溶液 [4.0mlの2メルカプトエタノール(14.2M)を蒸留水で17mlに希釈する](用時調製)
- C. 30mM MgCl₂溶液(610mgのMgCl₂・6H₂Oを約80mlの蒸留水に溶解後, 1.0N NaOHでpHを7.3に調整し, 蒸留水で100mlとする)
- D. 34mM ONPG溶液(205mgのONPGを20mlの試薬Aに攪拌溶解する)

酵素溶液: 酵素標品を予め氷冷した1.0mM MgCl₂, 0.1% BSAを含む50mMリン酸緩衝液, pH7.3で0.17~0.85U/mlに希釈する。

4. 手順

- ① 下記反応混液をキュベット(d=1.0cm)に調製し, 37°Cで約5分間予備加温する。

2.5ml	0.1Mリン酸緩衝液, pH7.3	(A)
0.1ml	メルカプトエタノール溶液	(B)
0.1ml	MgCl ₂ 溶液	(C)
0.2ml	ONPG溶液	(D)
- ② 酵素溶液0.1mlを添加し, ゆるやかに混和後, 水を対照に37°Cに制御させた分光光度計で410nmの吸光度変化を2~3分間記録し, その初期直線部分から1分間当りの吸光度変化を求める(Δ ODtest)。
- ③ 盲検は酵素溶液の代わりに酵素希釈液(1.0mMのMgCl₂を含む50mMリン酸緩衝液, pH 7.3)を0.1mlを加え, 上記同様に操作を行って1分間当りの吸光度変化を求める(Δ ODblank)。

5. 計算式

$$U/ml = \frac{\Delta OD/min (\Delta OD \text{ test} - \Delta OD \text{ blank}) \times 3.0(ml) \times \text{希釈倍率}}{3.5 \times 1.0 \times 0.1(ml)}$$

$$= \Delta OD/min \times 8.57 \times \text{希釈倍率}$$

3.5 : o-Nitrophenolの上記測定条件下でのミリ
モル分子吸光係数(cm²/micromole)

1.0 : 光路長(cm)