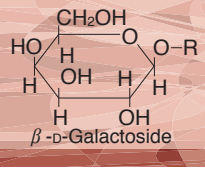
●TOYOBO ENZYMES●
(Diagnostic Reagent Grade)

β-GALACTOSIDASE
from Escherichia coli

β-D-Galactoside galactohydrolase (EC 3.2.1.23)



$+H_2O$ → D-Galactose + ROH

PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized.
Activity	: Grade Ⅱ 500U/mg-solid or more
Contaminants	: α-galactosidase $<1\times10^{-4}$%
	α-glucosidase $<1\times10^{-4}$%
	β-glucosidase $<2\times10^{-3}$%
	α-mannosidase $<1\times10^{-4}$%
	β-mannosidase $<1\times10^{-4}$%
	proteinasee <10mAbs/mg-P
Stabilizer	: Mg^{++}

PROPERTIES

Stability	: Stable at −20℃ for at least one year (Fig.1)
Molecular weight	: 540,000 [1,2]
Isoelectric point [3]	: 4.6
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^{280nm}_{1cm} (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℃ (Fig.3)
pH Stability	: pH 6.5−8.5 (25℃, 20hr) (Fig.4)
Thermal stability	: below 50℃ (pH 7.3, 15min) (Fig.5)
Substrate specificty	: This 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 Na_2HPO_4 and 0.1M KH_2PO_4 to reach pH 7.3 at 37℃.)

B. Mercaptoethanol solution : 3.36M ［Dilute 4.0ml of 2-mercaptoethanol (14.2M) to 17ml with H_2O.］ (Should be prepared fresh)

C. $MgCl_2$ solution : 30mM (Dissolve 610mg of $MgCl_2 \cdot 6H_2O$ in about 80ml of H_2O and, after adjusting the pH to 7.3 with 1.0 N NaOH, fill up to 100ml with H_2O.)

D. ONPG solution : 34mM (205mg ONPG/20ml of Reagent A)(Stable for one week if stored at 0－5℃)

E. Enzyme diluent : 50mM phosphate buffer, pH 7.3 contg. 1.0mM $MgCl_2$ and 0.1% BSA

Procedure

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

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)

Concentration in assay mixture	
Phosphate buffer	92 mM
ONPG	2.3mM
Mercaptoethanol	0.11 M
$MgCl_2$	1.0mM

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

   At the same time, measure the blank rate (ΔOD blank) by using the same method as the test except that the enzyme diluent (E) 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 :

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

Vt : Total volume (3.0ml)

Vs : Sample volume (0.1ml)

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

1.0 : Light path length (cm)

df : Dilution factor

REFERENCES

1) G.R.Graben, E.Steers, Jr.and C.B.Anfinsen; ***J.Biol.Chem., 240,*** 2468 (1965).
2) C.C.Contaxis and F.J.Reithel; ***Biochem,J., 124,*** 623 (1971).
3) K.Wallenfels and R.Weil; ***The Enzymes,Vol. 7, p.617*** (P.D.Boyer ed.), Academic Press. New York–London (1972).
4) A.Ulmann, M.E.Goldberg, D.Perrin and J.Monod; ***Biochemistry, 7,*** 261 (1968).
5) A.V.Fowler and I.Zabin; ***J.Biol.Chem., 245,*** 5032 (1970).
6) A.V.Fowler and I.Zabin; ***J.Biol.Chem., 247,*** 5425, 5432 (1972).
7) F.Melchers and W.Messer; Eur.***J.Biochem., 34,*** 228 (1973).
8) K.E.Langley, A.V.Fowler and I.Zabin; ***J.Biol.Chem., 250,*** 2587 (1975).

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℃ 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, CH_3CO; PCMB, p-Chloromercuribenzoate; MIA, Monoiodoacetate; NEM, N-Ethylmaleimide; IAA, Iodoacetamide; EDTA, Ethylenediaminetetraacetate; SDS, Sodium dodecyl sulfate; DAC, Dimethylbenzylalkylammonium chloride.

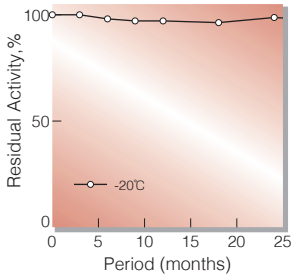


Fig.1. Stability (Powder form)
[kept under dry conditions]

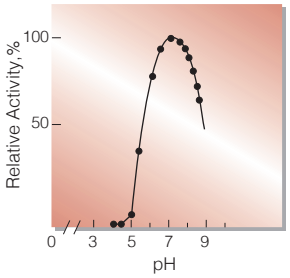


Fig.2. pH-Activity
[37℃,15 min-reaction in Britton-Robinson buffer]

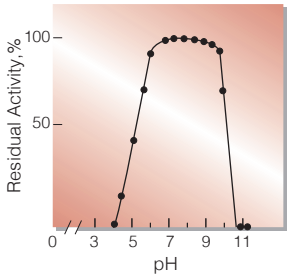


Fig.4. pH-Stability
[25℃,20hr-treatment with Britton-Robinson buffer]

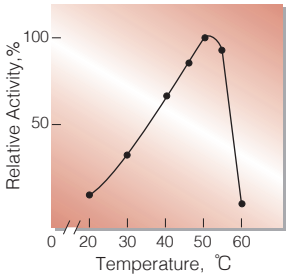


Fig.3. Temperature activity
[15min-reaction in 0.1M phospate buffer, pH7.3]

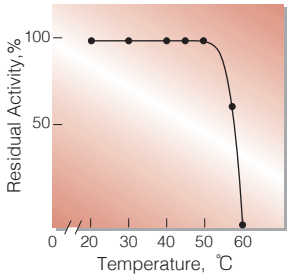


Fig.5. Thermal stability
[15min-treatment with 50mM phosphate buffer,pH7.3 contg. 1.0mM $MgCl_2$ enzyme concn.:80U/ml]

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℃)(0.1M Na_2HPO_4溶液と0.1M KH_2PO_4溶液を混合して37℃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 $MgCl_2$溶液(610mgの$MgCl_2・6H_2O$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 $MgCl_2$, 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℃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℃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(ΔODtest)。
③盲検は酵素溶液の代りに酵素希釈液(1.0mMの$MgCl_2$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(ΔODblank)。

5.計算式

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

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

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