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TECHNICAL INFORMATION

Catalog Number: 104939, 150039
beta-Galactosidase
Molecular Weight[1,2]: 540,000
Synonym: b-Galactoside galactohydrolase (EC 3.2.1.23)
Source: *Escherichia coli*
Reaction: b-D-Galactoside + H2O ----------> D-Galactose + Alcohol
Structure [4~8]: The enzyme is composed of four identical subunits having a molecular weight of ca. 135,000. The amino acid anlysis indicates approximately 1,170 residues per subunit.
Appearance: White to Off-White Powder
Activity: ≥300 u/mg solid (for cat #02150039) or ≥50 u/mg solid (for cat#02104939)
Contaminants: The preparation is practically free from other glycosidases (a-galactosidase, a-,b-glucosidase, a-,b -mannosidase, etc.) and proteinase.
Stability: Stable at 5ºC for at least 6 months.
Stabilizer: Mg^{++}
Isoelectric Point[3]: 4.61
Michaelis Constants: 3.0 x 10^{-4}M (o-Nitrophenyl-b-D-galactoside), 6.7 x 10^{-5}M (p-Nitro-phenyl- b-D-galactoside), 2.3 x 10^{-4}M (Phenyl-b-D-galactoside), 2.5 x 10^{-3}M (Lactose)
Inhibitors: p-Chloromercuribenzoate, Iodoacetamide, heavy metal ions (Zn^{++}, Fe^{++}, Zn^{++}, Cd^{++}, Cu^{++}, Pb^{++}, Ag^{+}, Hg^{++}), Ionic Detergents (SDS, DAC, etc.).
Optimum pH: 7.0 - 7.5
Optimum Temperature: 50 - 55ºC
pH Stability: pH 6.5 - 8.5 (25ºC, 20 hr)
Thermal Stability: Below 50ºC (pH 7.3, 15 min)
Substrate Specificity: The enzyme specifically hydrolyzes b-D-galactosyl linkage (Table 1).
Effect of Various Chemicals: (Table 2)
Applications: The enzyme is useful for the structure investigation of carbohydrate, the determination of lactose (foodstuff analysis) and as an enzyme label for enzyme immunoassay.
Principle: o-Nitrophenyl-b-D-galactopyranoside (ONPG) b-galactosidase> o-Nitrophenol (ONP) + D-Galactose. The appearance of o-nitrophenol is measured at 410 nm 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: 0.1 M (Prepare by mixing 0.1 M Na_2HPO_4 and 0.1 M KH_2PO_4 to reach pH 7.3 at 37ºC.)
B. Mercaptoethanol Solution: 3.36 M (Dilute 4.0 ml of 2-mercaptoethanol (14.2M) to 17 ml with H_2O).
C. $MgCl_2$ Solution: 30 mM (Disslve 610 mg of $MgCl_2.6H_2O$ in about 80 ml of H_2O and, after adjusting the pH to 7.3 with 1.0 N NaOH, fill up to 100 ml with H_2O.)
D. ONPG Solution: 34 mM (205 mg ONPG/20 ml of Reagent A) (Stable for one week if stored at 0 - 5ºC).
E. Enzyme diluent: 50 mM phosphate buffer, pH 7.3 contg. 1.0 mM $MgCl_2$
Procedure:
1. Prepare the following reaction mixture in a cuvette (d=1.0cm) and equilibrate at 37°C for about 5 minutes:

2.5 ml 0.1 M Phosphate buffer, pH 7.3 (A)
0.1 ml Mercaptoethanol solution (B)
0.1 ml $MgCl_2$ solution (C)
0.2 ml ONPG solution (D)

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

At the same time, measure the blank rate (DOD blank) by the same method as test except that the enzyme diluent is added instead of the enzyme solution.
Dilute the enzyme preparation to 0.17 - 0.83 U/ml with ice-cold enzyme diluent (E).

	Concentration in assay mixture

Phosphate buffer	92. mM
ONPG	2.3 mM
Mercaptoethanol	0.11 M
$MgCl_2$	1.0 mM

Calculation: Activity can be calculated by using the following formula:
Volume activity U/ml) = DOD/min (DOD test - DOD blank) x Vt x df = DOD/min x 8.57 x df
3.5 x 1.0 x Vs
Vt: Total volume (3.0 ml)
Vs: Sample volume (0.1 ml)
3.5: Millimolar extinction coefficient of ONP under the assay condition (cm_2/micromole)
1.0: Light path length (cm)
df: Dilution factor

Table 1, Substrate Specificity of b-Galactosidase

Substrate (2.3 mM)	Relative Activity	Vmax (Relative value)
o-Nitrophenyl-b-D- galactopyranoside	100	100
p-Nitrophenyl-b-D- galactopyranoside	14.7	13.4
Phenyl-b-D- galactohyranoside*	1.1	1.3
Lactose*	2.1	3.9
p-Nitrophenyl-a-D- galactopyranoside	0	0
p-Nitrophenyl-a-D- glucopyranoside	0	0
p-Nitrophenyl-b-D- glucopyranoside	0	0
p-Nitrophenyl-a-D- mannopyranoside	0	0
p-Nitrophenyl-b-D- mannopyranoside	0	0
p-Nitrophenyl-a-L- fucopyranocide	0	0
p-Nitrophenyl-b-L- fucopyranoside	0	0
p-Nitrophenyl-a-D- xylopyranoside	0	0
p-Nitrophenyl-b-D- xylopyranoside	0	0
	0	0

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

Table 2. Effect of Various Chemicals on b-Galactosidase (The enzyme dissolved in 50mM buffer, pH 7.0 (10 U/ml) was incubated with each chemical at 30ºC for 30 minutes. 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
$BaAc_2$		80	EDTA	5.0	103
$FeCl_3$		59	o-Phenanthroline	2.0	99
$CoCl_2$		83	a,a'-Dipyridyl	2.0	103
$MnCl_2$		100	Borate	50	98
$ZnSO_4$		6.2	NaF	2.0	99
$CdAc_2$		4.7	NaN_3	20	98
$NiCl_2$		77	Triton X-100	0.1%	101
$CuSO_4$		0.9	Brij 35	0.1%	103
$PbAc_2$		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		SDS	0.05%	75
Cystein	2.0	99	DAC	0.05%	0
PCMB	2.0	102			

0.3

Ac, CH3COO; PCMB, p-Chloromercuribenzoate; MIA, Monoiodoacetate; NEM, N-Ethylmaleimide; IAA, Iodoacetamide; EDTA, Ehtylendiaminetetraacetate; SDS, Sodium dodecyl sulfate; DAC, Dimethylbenzyl-alkyl-ammonium chloride.

Availability:

Catalog Number	Description	Size
104939	beta-Galactosidase, partially purified, lyophilized, activity approximately 50 units/mg minimum.	1 KU 5 KU 10 KU
150039	beta-Galactosidase, chromatographically purified, activity approximately > 300 units/mg.	1 KU 3 KU

Also Available:

Catalog Number	Description	Size
198930	beta-Galactosidase, from Trichoderma reesei, chromatographically purified, lyophilized, activity approximately 20 units/mg.	100 U
633631	Anti-Human beta-Galactosidase, monoclonal antibody	0.5 ml
55976	Rabbit Anti-beta-Galactosidase, polyclonal antibody	2 ml
56028	Goat anti-beta-Galactosidase, polyclonal antibody	1 ml
56030	Goat anti-beta-Galactosidase, polyclonal antibody, FITC conjugated	2 ml
56029	Goat anti-beta-Galactosidase, polyclonal antibody, HRP Conjugated	2 ml
191440	Sodium Phosphate Dibasic anhydrous ACS Reagent Grade	100 g 500 g 1 kg 5 kg
191430	Potassium Phosphate Monobasic, Anhydrous, ACS Reagent Grade	100 g 500 g 1 kg 5 kg
190242	2-Mercaptoethanol	100 ml 250 ml 500 ml 1 liter
191421	Magnesium Chloride, Hexahydrate, ACS Reagent Grade	100 g 500 g 1 kg 5 kg
1688145	1.0 N Sodium Hydroxide	10 ml
102473	o-Nitrophenyl-beta-D-galactopyranoside (ONPG)	500 mg 1 g 5 g 25 g

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