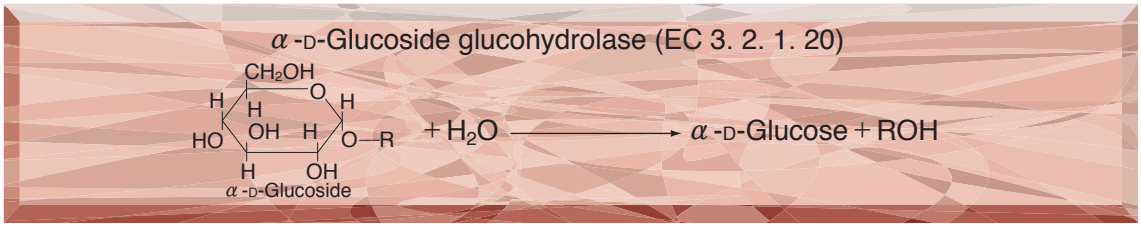
●TOYOBO ENZYMES●
(Diagnostic Reagent Grade)

α-GLUCOSIDASE(MALTASE)

from Microorganism



PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized
Activity	: Grade Ⅱ 20U/mg-solid or more
Contaminants	: α-amylase ≤1.0×10^{-5}%
Stabilizers	: BSA

PROPERTIES

Stability	: Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	: approx. 65,000 (Gel-filtration and SDS-PAGE)	
Isoelectric point	: 5.2	
Michaelis constant	: 6.3×10^{-4}M (p-Nitrophenyl-α-D-glucopyranoside)	
Inhibitors	: Ag^{+}, Hg^{++}, PCMB, MIA	
Optimum pH	: 6.0−7.0	(Fig.4)
Optimum temperature	: 60℃	(Fig.5)
pH Stability	: pH 5.0−9.0	(Fig.6)
Thermal stability	: below 60℃ (pH 7.0, 15min)	(Fig.7)

Substrate*	Relative hydrolysis rate**	Substrate*	Relative hydrolysis rate**
PNPG	100.0	Maltose	271.0
$PNPG_2$	205.0	Maltotriose	203.0
$PNPG_3$	284.0	Maltotetraose	168.0
$PNPG_5$	164.0	Maltopentaose	100.0

* : Substrate concn. 2.2mM

** : Glucose-forming activity, pH 6.8 at 37℃

Effect of various chemicals : (Table 1)

APPLICATIONS

This enzyme is useful for structural investigations of carbohydrates and for the enzymatic determination of α-amylase when coupled with hexokinase (HXK-311) and G-6-P dehydrogenase (G6D-311, G6D-321) in clinical analysis.

ASSAY

Principle:

p-Nitrophenyl-α-D-glucopyranoside (PNPG) $\xrightarrow{\alpha\text{-glucosidase}}$ p-Nitrophenol (PNP) + α-D-Glucose

The appearance of p-nitrophenol is measured at 400nm by spectrophotometry.

Unit definition:

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

Method:

Reagents

A. 0.1M Phosphate buffer, pH 7.0 (at 25℃)

B. PNPG solution : 20mM (603mg P-Nitrophenyl-α-D-glucopyranoside /100ml of H_2O)(Stable for two weeks if stored at 0−5℃)

C. Na_2CO_3 solution : 0.2M (21.2g Na_2CO_3/1,000ml of H_2O)

D. Enzyme diluent : 0.2M K-phosphate buffer, pH 7.0 containing 1mM of EDTA and 0.05% of Tween 20

Procedure

1. Prepare the following reaction mixture in a test tube and equilibrate at 37℃ for about 5 minutes.

1.0ml	0.1 M phosphate buffer	(A)
0.5ml	Substrate solution	(B)

Concentration in assay mixture		
Phosphate buffer	0.1	M
PNPG	5.0	mM
EDTA	0.25	mM
Tween 20	0.125	mg/ml

2. Add 0.5ml of the enzyme solution* and mix.
3. After exactly 15 minutes at 37℃, add 2.0ml of Na_2CO_3 solution (C) to stop the reaction and measure the optical density at 400nm against water (OD test).

   At the same time, prepare the blank by first mixing the reaction mixture with 2.0ml of Na_2CO_3 solution (C) after 15 min-incubation at 37℃, followed by the addition of the enzyme solution (OD blank).

* Dissolve the enzyme preparation in ice-cold enzyme diluent (D) and dilute to 0.006−0.022U/ml with the same buffer, immediately before assay.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta OD\ (OD\ test - OD\ blank) \times Vt \times df}{18.1 \times t \times 1.0 \times Vs} = \Delta OD \times 0.0295 \times df$$

Weight activity (U/mg) = (U/ml)×1/C

Vt : Total volume (4.0ml)

Vs : Sample volume (0.5ml)

18.1 : Millimolar extinction coefficient of p-nitrophenol under the assay condition (㎠/micromole)

1.0 : Light path length (cm)

t : Reaction time (15 minutes)

df : Dilution facter

C : Enzyme concentration in dissolution (c mg/ml)

REFERENCES

1) Y.Suzuki, M.Shinji and N.Eto; *Biochim.Biophys.Acta., 787,* 281 (1984).

2) Y.Takii, K.Daimon and Y.Suzuki; *Appl.Microbiol.Biotechnol., 38,* 243 (1992).

3) Y.Takii, K.Takahashi, K.Yamamoto, Y.Sogabe and Y.Suzuki; *Appl.Microbiol.Biotechnol., 44,* 629 (1996).

Table 1. Effect of Various Chemicals on α-Glucosidase

[The enzyme dissolved in 10mM phosphate buffer, pH 7.0 contg. 0.2% of BSA (5U/ml) was incubated with each chemical at 25℃ for 1hr.]

Chemical	Concn.(mM)	Residual activity(%)
None	—	100
Metal salt	2.0	
$MgSO_4$		97
$CaCl_2$		71
$Ba(OAc)_2$		106
$FeCl_2$		50
$CoCl_2$		63
$MnCl_2$		69
$ZnCl_2$		104
$CdCl_2$		47
$NiCl_2$		110
$CuSO_4$		39
$Pb(OAc)_2$		75
$AgNO_2$		0.3
$HgCl_2$		1.2
2-Mercaptoethanol	2.0	111
PCMB	1.0	1.3
MIA	2.0	0.8
NEM	2.0	120
IAA	2.0	106
Hydroxylamine	2.0	115
EDTA	5.0	112
o-Phenanthroline	2.0	114
α,α′-Dipyridyl	1.0	122
Borate	50	119
NaF	2.0	118
NaN_3	2.0	123
Triton X-100	0.10%	123
Brij 35	0.10%	121
Tween 20	0.10%	124
Span 20	0.10%	43
Na-cholate	0.10%	102
SDS	0.05%	10
DAC	0.05%	124

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

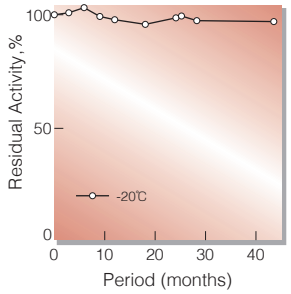


Fig.1. Stability (Powder form)

〔kept under dry conditions〕

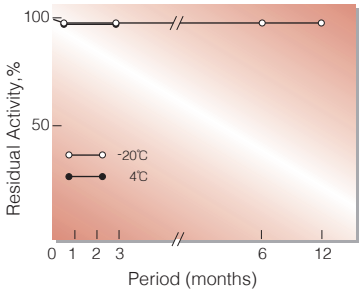


Fig.2. Stability (Powder form)

〔kept under dry conditions〕

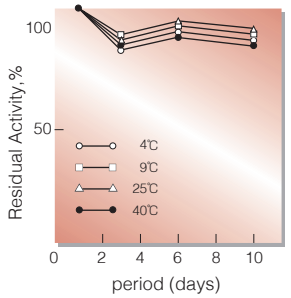


Fig.3. Stability (Liquid form)

〔in 50mM PIPES buffer solution, pH7.0 (contg. 0.5mM $CaCl_2$, 0.1% detergent) enzyme concn,:5U/ml〕

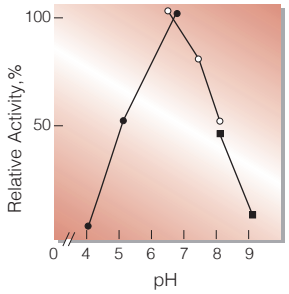


Fig.4. pH-Activity

〔37℃, 15 min-reaction in 100mM buffer solution: ●, pH4.0-6.0 acetate ; O, pH6.0-8.0, phosphate; ■, pH8.0-9.0, borate

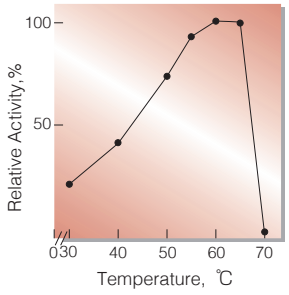


Fig.5. Thermal activity

〔15 min-reaction in 100mM phosphate buffer, pH7.0〕

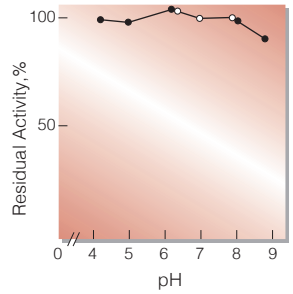


Fig.6. pH-Stability

〔25℃, 20hr-treatment with 50mM buffer solution contg; 0.2% of BSA: ●, pH4.0-6.0 acetate: ○, pH6.0-8.0, phosphate; ■, pH8.0-9.0, borate. enzyme concn. : 5U/ml〕

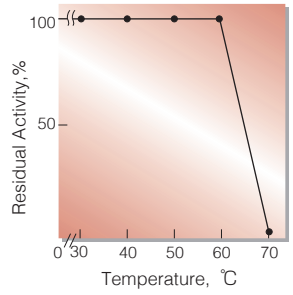


Fig.7. Thermal stability

〔15min-treatment with 0.2M K-phosphate buffer, pH7.0 contg. 1mM EDTA and 0.05% Tween20. enzyme concn.: 5U/ml〕

活性測定法（Japanese）

1.原理

p-Nitrophenyl-α-D-glucopyranoside (PNPG)
$\xrightarrow{\alpha\text{-glucosidase}}$ p-Nitrophenol＋α-D-Glucose
p-Nitrophenolの生成量を400nmの吸光度変化で測定する。

2.定義

下記条件下で1分間に1マイクロモルのp-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, pH 7.0 (25℃)
B. 20mM PNPG水溶液〔603mgのP-ニトロフェニル-α-D-グルコピラノシドを100mℓの蒸留水に攪拌溶解する〕(1～5℃保存で2週間は使用可能)
C. 0.2M Na_2CO_3溶液(21.2gの無水炭酸ナトリウムを蒸留水に溶解し1,000mℓとする)
酵素溶液：酵素標品を予め氷冷した1mM EDTA・2Naと0.05%Tween20を含む0.2Mリン酸緩衝液,pH7.0で溶解し0.006～0.022U/mℓに希釈する。

4.手順

①試験管に下記反応混液を調製し,37℃で約5分間予備加温する。
1.0mℓ　リン酸緩衝液, pH 7.0　(A)
0.5mℓ　基質溶液　(B)
②酵素溶液を0.5mℓを加え,反応を開始する。
③37℃で正確に15分間反応させた後,Na_2CO_3溶液(C) 2.0mℓ加えて反応を停止させる。この液につき400nmにおける吸光度を測定する(OD test)。
④盲検は反応混液①を37℃で15分間放置後,Na_2CO_3溶液(C) 2.0mℓを加えて混和し,次いで酵素溶液0.5mℓを加えて調整する。以下同様に吸光度を測定する(ODblank)。

5.計算式

$$U/m\ell = \frac{\Delta OD\ (OD\ test - OD\ blank) \times 4.0(m\ell) \times 希釈倍率}{18.1 \times 1.0 \times 15(分) \times 0.5(m\ell)}$$
$$= \Delta OD \times 0.0295 \times 希釈倍率$$
$$U/mg = U/m\ell \times 1/C$$

18.1　：p-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)
C　：溶解時の酵素濃度(c mg/mℓ)