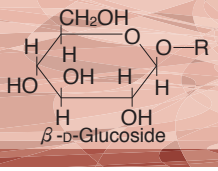
●TOYOBO ENZYMES●
(Diagnostic Reagent Grade)

β-GLUCOSIDASE
from Sweet almond

β-D-Glucoside glucohydrolase (EC 3. 2. 1. 21)



$+H_2O$ → β-D-Glucose + ROH

PREPARATION and SPECIFICATION

Appearance	: Light yellow amorphous powder, lyophilized
Activity	: Grade Ⅱ 10U/mg-solid or more (containing approx. 50% of BSA)
Contaminant	: α-Amylase ≤5.0×10^{-4}%
Stabilizers	: BSA, glutathione (reduced)

PROPERTIES

Stability	: Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	: approx. 110,000	
Isoelectric point	: 7.3 [1)]	
Michaelis constants	: 2.8×10^{-3}M (p-Nitrophenyl-β-D-glucopyranoside), 3.3×10^{-3}M (2,4-Dichlorophenyl-β-D-glucopyranoside)	
Structure	: 2 subunits per enzyme molecule	
Optimum pH	: 5.5	(Fig.4)
Optimum temperature	: 50−55℃	(Fig.5)
pH Stability	: pH 6.0−9.0 (25℃, 64hr)	(Fig.6)
Thermal stability	: below 50℃ (pH 7.3, 1hr)	(Fig.7)
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 α-glucosidase (AGH-211) in clinical analysis.

ASSAY

Principle:

p-Nitrophenyl-β-D-glucopyranoside (PNPG) $\xrightarrow{\beta\text{-glucosidase}}$ p-Nitrophenol (PNP) + 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. Acetate buffer, pH 5.0 (at 25℃) : 0.1M

B. PNPG solution : 20mM (603mg p-nitrophenyl-β-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 : 10mM phosphate buffer, pH 7.0 containing 0.2% of BSA.

Procedure

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

1.0ml	0.1M Acetate buffer, pH 5.0	(A)
0.5ml	Substrate solution	(B)

Concentration in assay mixture		
Acetate buffer	50	mM
PNPG	5.0	mM
BSA	0.05	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 50mM Tris-HCl buffer pH 7.8 (ca. 1mg/ml) and dilute to 0.006−0.022U/ml with the enzyme diluent (D), immediately before assay.

Calculation

Activity can be calculated by using the following formula :

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

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 factor

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

REFERENCES

1) A.K.Grover, D.D.Macmurchie and R.J.Cushley; ***Biochim.Biophys.Acta, 482,*** 98 (1977).
(Characteristics of β-Glucosidase from almond)

2) R.Heyworth and P.G.Walker; ***Biochem.J., 83,*** 331 (1962).

3) J.H.Hash and K.W.King; ***J.Biol.Chem., 232,*** 395 (1958).

Table 1. Effect of Various Chemicals on β-Glucosidase [Residual activity after 1 hr-treatment at 30℃.]

Chemical	Concn.(mM)	Residual activity(%)
None	—	100
Metal salt	0.5	
$CaCl_2$		92.7
$FeSO_4$		94.1
$CoCl_2$		95.5
$ZnCl_2$		95.0
$CuSO_4$		94.5
$HgCl_2$		99.8
$CrCl_2$		93.9
$MgSO_4$		96.8
$SnCl_2$		93.6
$CdCl_2$		93.0
$AgNO_3$		92.7
$NiCl_2$		95.5
$MnCl_2$		94.3
$BaCl_2$		93.9
$FeCl_3$		99.8
o-Phenanthroline	0.5	94.3
α,α′-Dipyridyl	0.5	94.3
Borate	25	94.1
PCMB	0.05	94.5
MIA	0.5	89.3
NaF	0.5	96.6
NaN_3	10	98.9
EDTA	5.0	96.1
Triton X-100	0.5%	102.3
Na-cholate	0.5%	99.5

PCMB, p-Chloromercuribenzoate; MIA, Monoiodoacetate; EDTA, Ethylenediaminetetraacetate.

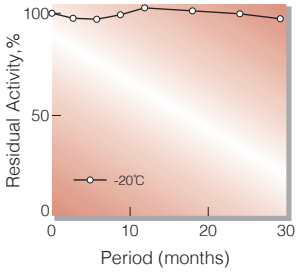


Fig.1. Stability (Powder form)
〔kept under dry conditions〕

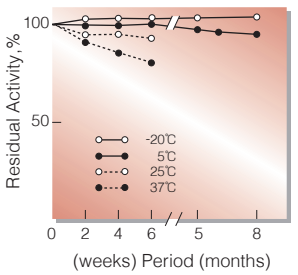


Fig.2. Stability (Powder form)
〔kept under dry conditions〕

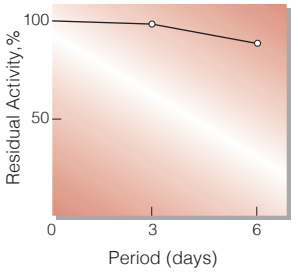


Fig.3. Stability (Liquid form at 25℃)
〔enzyme concentration: 1.0mg/ml
buffer composition: 50mM Tris-HCl buffer, pH7.8〕

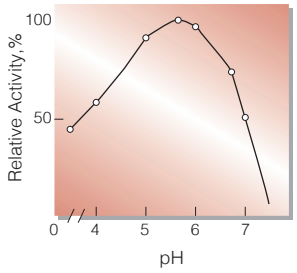


Fig.4. pH-Activity
〔37℃.15 min-reaction in 50mM acetate buffer.〕

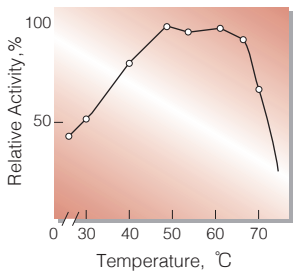


Fig.5.Temperature activity
〔15 min-reaction in 50mM acetate buffer, pH5.0〕

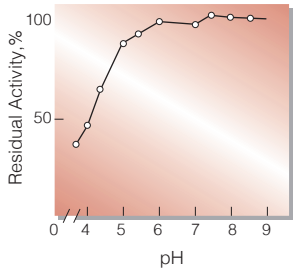


Fig.6. pH-Stability
〔25℃, 64hr-treatment with 50mM buffer solution:pH3.5-6.0, acetate; pH6.5-9.0, Tris-HCl〕

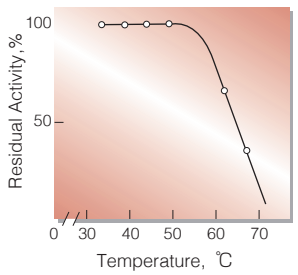


Fig.7. Thermal stability
〔1hr-treatment with 50mM Tris-HCl buffer,pH7.3.〕

活性測定法（Japanese）

1.原理

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

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酢酸緩衝液, pH5.0(25℃)
B. 20mM PNPG水溶液(603mgのP-ニトロフェニル-β-D-グルコピラノシドを100mℓの蒸留水に攪拌溶解する)(0～5℃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50mM Tris-HCl緩衝液pH7.8で約1mg/mℓに溶解し,分析直前に0.2%牛血清アルブミン(BSA)を含む10mMリン酸緩衝液，pH7.0で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	0.1M酢酸緩衝液, pH5.0	(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を加えて調製する。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/mℓ×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)