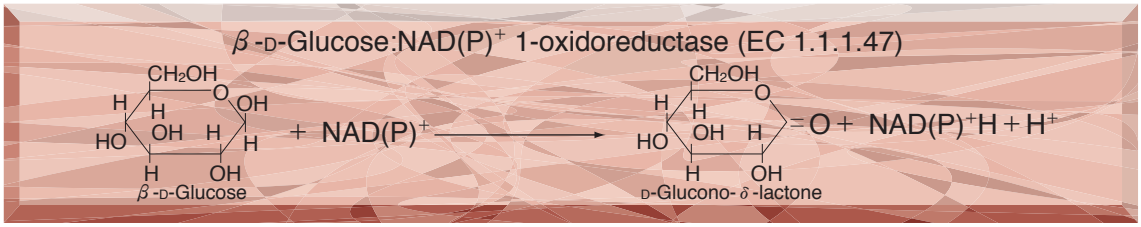
●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

GLUCOSE DEHYDROGENASE

(NAD(P)-dependent)

from Microorganism



PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized	
Activity	: Grade Ⅲ 250U/mg-solid or more	
Contaminants	: NADH oxidase	≤1.0×10^{-3}%
	α-Glucosidase	≤1.0×10^{-3}%
	Glucose-6-phosphate dehydrogenase	≤1.0×10^{-3}%

PROPERTIES

Stability	: Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	: approx. 101,000 (Gel filtration)	
Isoelectric point	: 4.5	
Michaelis constants	: NAD^+linked : 1.38×10^{-2}M (D-Glucose) 3.09×10^{-4}M (NAD^+)	
	$NADP^+$linked : 1.25×10^{-2}M (D-Glucose) 4.07×10^{-5}M ($NADP^+$)	
Inhibitors	: Ag^+, Hg^{2+}, Monoiodoacetate	
Optimum pH	: 9.0	(Fig.4)
Optimum temperature	: 55℃	(Fig.5)
pH Stability	: pH 6.0−7.5 (20℃, 16hr)	(Fig.6)
Thermal stability	: 45℃ (15min-treatment with 50mM K-phosphate buffer, pH 7.0)	(Fig.7)
Substrate specificity	: Specific for β-D-Glucose or 2-Deoxy-glucose (Table.1)	
	(Either NAD^+ or $NADP^+$ serves as coenzyme.)	

APPLICATIONS

This enzyme is useful for enzymatic determination of D-Glucose.

ASSAY

Principle:

β-D-Glucose + NAD^+ $\xrightarrow{\text{glucose dehydrogenase}}$ D-Glucono-δ-lactone + NADH + H^+

The appearance of NADH is measured at 340nm by spectrophotometry.

Unit definition:

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

Method:

Reagents

A. Tris-HCl buffer, pH 8.0 : 0.1M
B. D-Glucose solution : 1.5M
C. β-NAD^+ solution : 80mg/ml
D. Enzyme diluent : 50mM K-phosphate buffer, pH 7.0 contg. 0.1% BSA

Procedure

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

2.6ml	Tris-HCl buffer, pH 8.0	(A)
0.3ml	Substrate solution	(B)
0.1ml	β-NAD^+ solution	(C)

Concentration in assay mixture	
Tris-HCl buffer	85.25mM
D-Glucose	147.54mM
NAD^+	3.66mM

2. Add 0.05ml of the enzyme solution* and mix by gentle inversion.
3. Record the increase in optical density at 340nm against water for 2 to 5 minutes in a spectrophotometer thermostated at 37℃, and calculate the 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 (D) is added instead of the enzyme solution.

* Dissolve the enzyme preparation in ice-cold enzyme diluent (D), dilute to 0.8−1.2U/ml with the same buffer and store on ice.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta\text{OD/min}\ (\Delta\text{OD test} - \Delta\text{OD blank}) \times \text{Vt} \times \text{df}}{6.22 \times 1.0 \times \text{Vs}} = \Delta\text{OD/min} \times 9.807 \times \text{df}$$

Weight activity (U/mg) = (U/ml) × 1/C

Vt : Total volume (3.05ml)
Vs : Sample volume (0.05ml)
6.22 : Millimolar extinction coefficient of NADH under the assay conditions (㎤/micromole)
1.0 : Light path length (cm)
df : Dilution factor
C : Enzyme concentration in dissolution (c mg/ml)

Table 1. Substrate Specificity of Glucose dehydrogenase

Substrate (150mM)	Relative activity(%)	Substrate (150mM)	Relative activity(%)
D-Glucose	100.0	Galactose	1.7
L-Glucose	0.0	D-Lactose	1.5
D-Xylose	16.2	D-Sorbitole	0.0
2-Deoxy-glucose	127.0	D-Mannitol	0.0
L-Sorbose	0.0	Sucrose	0.0
D-Mannose	5.1	Inositol	0.0
D-Fructose	0.0	Maltose	1.4

Table 2. Effect of Various Chemicals on Glucose dehydrogenase

[The enzyme dissolved in 50mM K-phosphate buffer, pH 7.0 (2.8U/ml) was incubated with each chemical for 1hr at 30℃.]

Chemical	Concn.(mM)	Residual activity(%)	Chemical	Concn.(mM)	Residual activity(%)
None	—	100	KF	2.0	98.7
Metal salt	2.0		NaF	10.0	100.6
$AgNO_3$		7.1	NaN_3	20.0	101.6
$Ba(OAc)_2$		98.2	NEM	2.0	97.6
$CaCl_2$		98.9	MIA	2.0	0.4
$Cd(OAc)_2$		96.6	IAA	2.0	92.2
$CoCl_2$		96.4	EDTA	5.0	107.2
$CuSO_4$		99.5	$(NH_4)_2SO_4$	20.0	96.0
$FeCl_3$		98.1	Borate	20.0	101.4
$FeSO_4$		96.6	o-Phenanthroline	2.0	97.7
$HgCl_2$		5.9	α,α'-Dipyridyl	1.0	100.3
$MgCl_2$		101.5	Urea	2.0	122.5
$MnCl_2$		100.9	Guanidine	2.0	99.2
$NiCl_2$		93.4	Hydroxylamine	2.0	107.2
$Pb(OAc)_2$		99.8			
$ZnSO_4$		102.1			

Ac, CH_3CO; NEM, N-Ethylmaleimide; MIA, Monoiodoacetate; IAA, Iodoacetamide; EDTA, Ethylenediaminetetraacetate.

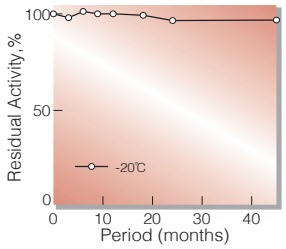


Fig.1. Stability (Powder form)

〔kept under dry conditions〕

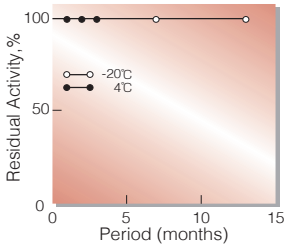


Fig.2. Stability (Powder form)

〔kept under dry conditions〕

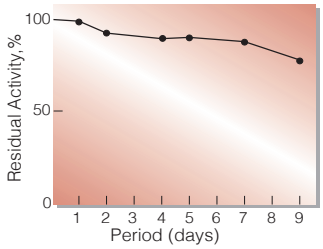


Fig.3. Stability (Liquid form)

〔25℃,in 83mM Tris-HCl buffer solution
pH8.0(contg.3.7mM β-NAD,40U/ml mutarotase)
enzyme concn.:300U/ml〕

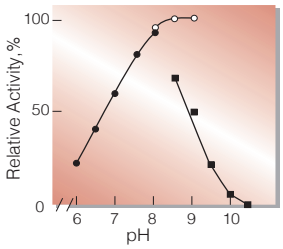


Fig.4. pH-Activity

〔37℃,5min-reaction in 80mM buffer solution
●:pH6.0-8.0 K-phosphate
○:pH8.0-9.0,Tris-HCl
■:pH8.5-10.5 Carbonate〕

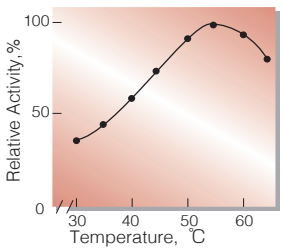


Fig.5. Temperature activity

〔in 80 mM Tris-HCl buffer, pH8.0〕

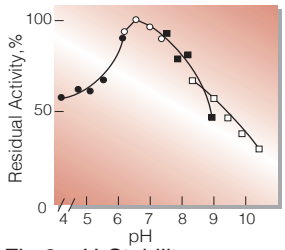


Fig.6. pH-Stability

〔20℃,16hr with 0.1M buffer solution
●:pH4.0-6.0 acetate
○:pH6.0-8.0 K-phosphate
■:pH7.5-9.0 Tris-HCl
□:pH8.5-10.5 carbonate
enzyme concn.:10U/ml〕

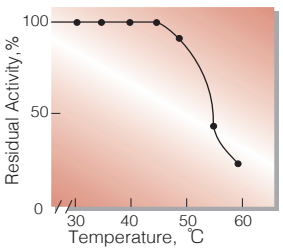


Fig.7. Thermal stability

〔15min-treatment with 50mM K-phosphate buffer pH7.0 enzyme concn.: 12U/ml〕

活性測定法（Japanese）

1.原理

β-D-Glucose＋NAD^+ $\xrightarrow{\text{glucose deyhydrogenase}}$ D-Glucono-δ-lactone＋NADH＋H^+

NADHの生成量を340nm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NADHを生成する酵素量を1単位 (U)とする。

3.試薬

A.　0.1M　　Tris-HCl緩衝液,　pH8.0
B.　1.5M　　D-Glucose水溶液
C.　80㎎/㎖NAD^+水溶液 (用時調製)
酵素溶液：酵素標品を予め氷冷した0.1%BSAを含む50mMK-リン酸緩衝液,pH 7.0で溶解し,分析直前に同緩衝液で0.8～1.2U/㎖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。

2.6㎖	Tris-HCl緩衝液	(A)
0.3㎖	D-グルコース水溶液	(B)
0.1㎖	NAD^+水溶液	(C)

②酵素溶液0.05㎖を添加し,ゆるやかに混和後,水を対照に37℃に制御された分光光度計で340nmの吸光度変化を5分間記録し, その2～5分の直線部分から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わりに酵素希釈液(リン酸緩衝液,pH 7.0)を0.05㎖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

$$U/㎖ = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times 3.05 \times 希釈倍率}{6.22 \times 1.0 \times 0.05}$$

$$= \Delta OD/min \times 9.807 \times 希釈倍率$$

U/mg ＝U/㎖×1/C
6.22　：NADH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 ㎎/㎖)