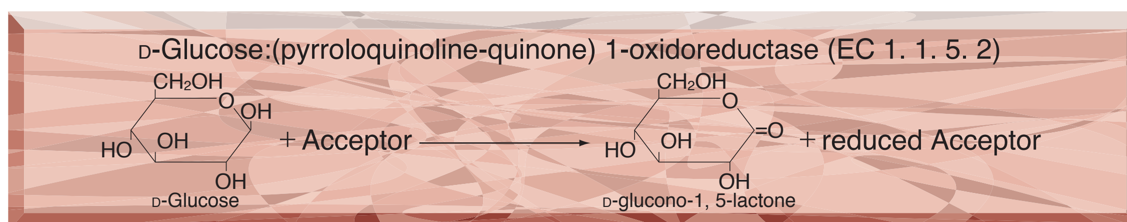
●TOYOBO ENZYMES●
(Diagnostic Reagent Grade)

GLUCOSE DEHYDROGENASE (PQQ-dependent)

from Microorganism



PREPARATION and SPECIFICATION

Appearance	: Purple amorphous powder, lyophilized
Activity	: Grade Ⅲ 500U/mg-solid or more
Contaminants	: Glucose dehydrogenase (NAD-dependent) ≤1.0×10^{-3}%
	Hexokinase ≤1.0×10^{-3}%
Stabilizers	: Ca^{++}, BSA

PROPERTIES

Stability	: Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	: approx. 100,000 (by gel filtration)	
Michaelis constant	: 4.8mM (D-Glucose)	
Inhibitors	: Cu^{++}, Pb^{++}, Ag^{+}	
Optimum pH	: 7.0	(Fig.2)
Optimum temperature	: 37℃	(Fig.3)
pH Stability	: pH 3.5−8.5 (25℃, 16hr)	(Fig.4)
Thermal stability	: below 50℃ (pH 7.5, 30min)	(Fig.5)
Substrate specificity	: (Table 1)	
Effect of various chemicals	: (Table 2)	

APPLICATIONS

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

ASSAY

Principle:

D-glucose＋PMS —(PQQ-glucose dehydrogenase)→ D-glucono-1, 5-lactone＋PMS (red)

2PMS (red)＋NTB ——→ 2PMS＋Diformazan

The appearance of diformazan formed by the reduction of nitrotetrazorium blue (NTB) with phenazine methosulfate (PMS)(red) is measured at 570nm by spectrophotometry.

Unit definition:

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

Method:

Reagents

A. D-Glucose solution : 1M [1.8g D-Glucose (MW＝180.16)/10ml H_2O] keep this solution at room temperature at least 3 hours before use

B. PIPES-NaOH buffer, pH 6.5 : 50mM [Weight 1.51g of PIPES (MW＝302.36), suspended in 60ml of H_2O, dissolve with 5N NaOH and add 2.2ml of 10% Triton X-100. After adjusting pH to 6.5±0.05 at 25℃ with 5N NaOH, fill up to 100ml with H_2O]

C. PMS solution : 3.0mM [9.19mg Phenazine methosulfate (MW＝306.34)/10ml H_2O]

D. NTB solution : 6.6mM [53.96mg nitrotetrazorium blue (MW＝817.65)/10ml H_2O]

E. Enzyme diluent : 50mM PIPES-NaOH buffer, pH 6.5 containing 1mM $CaCl_2$, 0.1% Triton X-100, 0.1% BSA

Procedure

1. Prepare the following reaction mixture in a brownish bottle and store on ice. (Prepare freshly)

0.9ml	D-Glucose solution	(A)
25.5ml	PIPES-NaOH buffer, pH 6.5	(B)
2.0ml	PMS solution	(C)
1.0ml	NTB solution	(D)

Concentration in assay mixture	
PIPES-buffer	42 mM
D-Glucose	30 mM
PMS	0.20mM
NTB	0.22mM

2. Pipet 3.0ml of working solution into a test tube (plastic tube) and equilibrate at 37℃ for about 5 minutes.
3. Add 0.1ml of enzyme solution* and mix by gentle inversion.
4. Record the increase of optical density at 570nm against water for 4 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 the same method as test except that the enzyme diluent (E) is added instead of the enzyme solution.

* Dissolve the enzyme preparation on ice cold enzyme diluent (E) and dilute to 0.1−0.8U/ml with the same buffer, immediately before assay. (The use of plastic tube is recommended because of sticky nature.)

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times Vt \times df}{20.1 \times 1.0 \times Vs} = \Delta OD \times 1.54 \times df$$

Weight activity (U/mg) ＝ (U/ml)×1/C

Vt : Total volume (3.1ml)

Vs : Sample volume (0.1ml)

20.1 : Half a millimolar extinction coefficient of diformazan (㎠/0.5 micromole)

1.0 : Light path length (cm)

df : Dilution factor

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

REFERENCES

1) K.Matsushita et al.; *FEMS Microbiology Letters, 55,* 53 (1988).

Table 1. Substrate Specificity of PQQ-Glucose dehydrogenase

Substrate (50mM)	Relative activity(%)	Substrate (50mM)	Relative activity(%)
D-Glucose	100.0	Galactose	16.0
L-Glucose	0.3	D-Lactose	68.9
D-Xylose	15.0	D-Sorbitole	0.2
2-Deoxy-glucose	4.9	D-Mannitol	0.0
L-Sorbose	0.5	Sucrose	0.2
D-Mannose	10.8	Inositol	0.0
D-Fructose	0.3	Maltose	107.0

Table 2. Effect of Various Chemicals on PQQ-Glucose dehydrogenase

[The enzyme dissolved in 50mM PIPES-NaOH buffer, pH 6.5 contg. 1mM $CaCl_2$, 0.1% Triton X-100 (5U/ml) was incubated with each chemical at 25℃ for 1hr.]

Chemical	Concn.(mM)	Residual activity(%)
None	—	100
Metal salt	2.0	
$MgSO_4$		108
$CaCl_2$		108
$Ba(OAc)_2$		105
$FeCl_3$		79
$CoCl_2$		42
$MnCl_2$		105
$ZnCl_2$		45
$Cd(OAc)_2$		107
$NiCl_2$		101
$CuSO_4$		0
$Pb(OAc)_2$		0
$AgNO_3$		0
$HgCl_2$		77
2-Mercaptoethanol	2.0	99
PCMB	1.0	97

Chemical	Concn.(mM)	Residual activity(%)
MIA	2.0	87
NEM	2.0	100
IAA	2.0	98
Hydroxylamine	2.0	19
EDTA	5.0	79
O-Phenanthroline	2.0	7
α,α'-Dipyridyl	1.0	103
Borate	5.0	110
NAF	2.0	111
NaN_3	2.0	115
Triton X-100	0.10%	101
Brij 35	0.10%	22
Tween 20	0.10%	104
Span 20	0.10%	60
Na-Cholate	0.10%	67
SDS	0.05%	33
DAC	0.05%	113

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

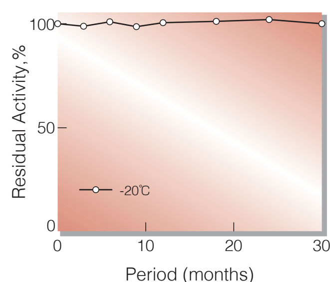


Fig.1. Stability (Powder form)
〔kept under dry conditions〕

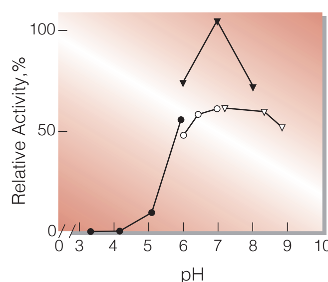


Fig.2. pH-Activity
〔25℃, in 50mM buffer solution; ●—●, acetate; ▼—▼, phosphate; ○—○, PIPES; ▽—▽, Tris-HCl.〕

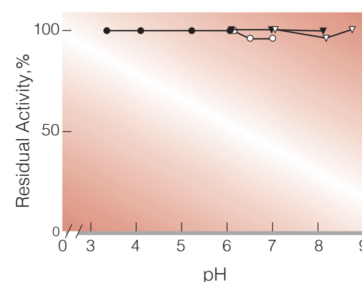


Fig.4. pH-Stability
〔25℃, 16 hr-treatment with 50mM buffer solution contg. 1mM $CaCl_2$; ●—●, acetate; ▼—▼, phosphate; ○—○, PIPES; ▽—▽, Tris-HCl.〕

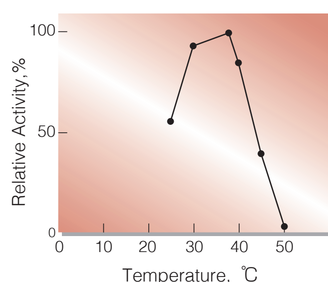


Fig.3. Temperature Activity
〔in 42mM PIPES-NaOH buffer, pH 6.5〕

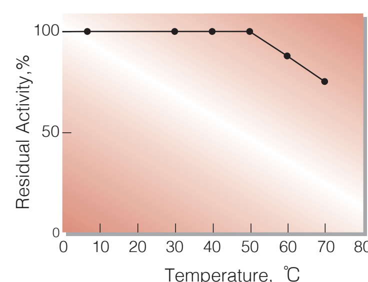


Fig.5. Thermal stability
〔30min.-treatment with 50mM PIPES-NaOH buffer, pH 6.5 contg. 1mM $CaCl_2$ enzyme concentration: 5.0 U/ml〕

活性測定法（Japanese）

1.原理

D-Glucose＋PMS —PQQ-glucose dehydrogenase→
D-glucono-1, 5-lactone＋PMS(red)
2PMS(red)＋NTB ——→ 2PMS＋Diformazan
PMS(phenazine methosulfate)を介してNTB (nitrotetrazorium blue)を還元し,生成したdiformazanの570nmにおける吸光度を測定する。

2.定義

下記条件下で1分間あたり1/2マイクロモルのdiformazanを生成する酵素量を1単位(U)とする。

3.試薬

A. 1M D-グルコース溶液(1.8gのD-グルコースを蒸留水に溶解し,10mℓとし,室温で3時間経過したものを使用する)
B. 50mM PIPES-NaOH緩衝液,pH6.5(1.51gのPIPESを60mℓの蒸留水に懸濁し,5N NaOHで溶解後,10%のTritonX-100溶液2.2mlを加える。5N NaOHを使って25℃でpHを6.5±0.05に調整後,蒸留水で100mℓとする)
C. 3mM PMS溶液(9.19mgのPMSを10mℓの蒸留水に溶解する)
D. 6.6mM NTB溶液(53.96mgのNTBを10mℓの蒸留水に溶解する)
酵素溶液：酵素標品を予め氷冷した1mM $CaCl_2$と0.1% TrironX-100と0.1%BSAを含む50mM PIPES-NaOH緩衝液,pH6.5で0.1～0.8U/mℓに希釈する。

4.手順

①褐色瓶中に下記反応混液を調製し,氷冷保存する。(用時調製)

0.9mℓ	D-グルコース溶液	(A)
25.5mℓ	PIPES-NaOH緩衝液	(B)
2.0mℓ	PMS溶液	(C)
1.0mℓ	NTB溶液	(D)

②反応混液3.0mℓをプラスチック製のキュベット(d＝1.0cm)に分注し37℃で約5分間予備加温する。
③酵素溶液を0.1mℓを添加し,緩やかに混和後,水を対照に37℃に制御された分光光度計で570nmの吸光度変化を4～5分間記録し,その直線部分から1分間あたりの吸光度変化を求める(ΔODtest)。
④盲検は反応混液①に酵素液の代りに酵素希釈液(1mM $CaCl_2$と0.1% TritonX-100と0.1% BSAを含む50mM PIPES-NaOH緩衝液, pH6.5)0.1mℓを加え,上記同様に操作を行い,1分間当たりの吸光度変化を求める(ΔODblank)。

5.計算式

$$U/m\ell = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times 3.1 \times df}{20.1 \times 1.0 \times 0.1}$$

$$= \Delta OD/min \times 1.54 \times df$$

$$U/mg = U/m\ell \times 1/C$$

20.1 ：diformazanの1/2ミリモル分子吸光係数(㎠/0.5 micromole)
1.0 ：光路長(cm)
C ：溶解時の酵素濃度(C mg/mℓ)

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