Recombinant Glucose Dehydrogenase (GDH-PQQ)

G1492997

Storage temperature: -20℃. Avoid freeze/thaw cycle.

Introduction:

In enzymology, a quinoprotein glucose dehydrogenase (EC 1.1.5.2) is an enzyme that catalyzes the chemical reaction: D-glucose + ubiquinone ↔D-glucono-1,5-lactone + ubiquinol. Thus, the two substrates of this enzyme are D-glucose and ubiquinone, whereas its two products are D-glucono-1,5-lactone and ubiquinol. This enzyme belongs to the family of oxidoreductases, specifically those acting on the CH-OH group of donor with a quinone or similar compound as acceptor. This enzyme participates in pentose phosphate pathway. It employs one cofactor, PQQ.

D-Glucose + Acceptor → D-glucono-1,5-lactone + reduced Acceptor

PREPARATION and SPECIFICATION

Appearance	Purple amorphous powder, lyophilized
Activity	≥500U/mg powder
Contaminants	Glucose dehydrogenase (NAD-dependent) ≤1.0×10^{-3}%; Hexokinase ≤1.0×10^{-3}%
Stabilizers	Ca^{2+},BSA

PROPERTIES

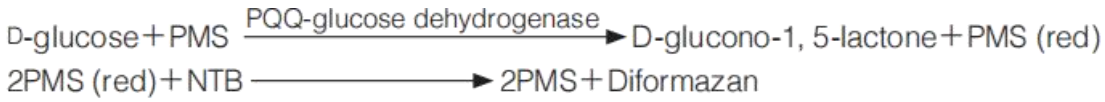
Stability	Stable at -20℃ for at least one year	Fig.1
Molecular weight	approx. 100,000 (by gel filtration)	
Michaelis constant	4.8mM (D-Glucose)	
Inhibitors	Cu^{2+}, Pb^{2+}, Ag^{+}	
Optimum pH	7.0	Fig.2
Optimum temperature	37℃	Fig.3
pH Stability	pH 3.5−8.5 (25℃, 16hr)	Fig.4
Thermal stability	below 50℃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



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	42mM
D-Glucose	30mM
NTB	0.20mM
PMS	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:

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

$$\text{Weight activity (U/mg)} = (\text{U/ml}) \times 1/\text{C}$$

Vt	Total volume (3.1ml)
Vs	Sample volume (0.1ml)
20.1	Half a millimolar extinction coefficient of diformazan (cm²/0.5 micromole)
1.0	Light path length (cm)
df	Dilution factor
C	Enzyme concentration in dissolution (c mg/ml)

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)	Activity(%)	Chemical	Concn.(mM)	Activity(%)
None	-	100	MIA	2.0	87
Metal salt	2.0		NEM	2.0	100
$MgSO_4$		108	IAA	2.0	98
$CaCl_2$		108	Hydroxylamine	2.0	19
$Ba(OAc)_2$		105	EDTA	5.0	79
$FeCl_3$		79	O-Phenanthroline	2.0	7
$CoCl_2$		42	α,α′-Dipyridyl	1.0	103

$MnCl_2$		105	Borate	5.0	110
$ZnCl_2$		45	NAF	2.0	111
$Cd(OAc)_2$		107	NaN_3	2.0	115
$NiCl_2$		101	Triton X-100	0.10%	101
$CuSO_4$		0	Brij 35	0.10%	22
$Pb(OAc)_2$		0	Tween 20	0.10%	104
$AgNO_3$		0	Span 20	0.10%	60
$HgCl_2$		77	Na-Cholate	0.10%	67
2-Mercaptoethanol	2.0	99	SDS	0.05%	33
PCMB	1.0	97	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.

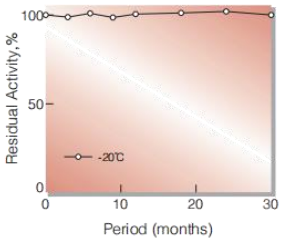


Fig.1. Stability (Powder form)
〔kept under dry conditions〕

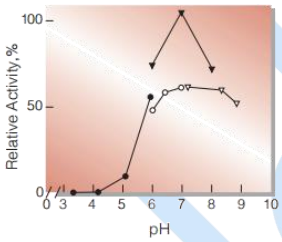


Fig.2. pH-Activity
〔25℃, in 50mM buffer solution; ●—●, acetate; ▼—▼, phosphate; ○—○, PIPES; ▽—▽, Tris-HCl.〕

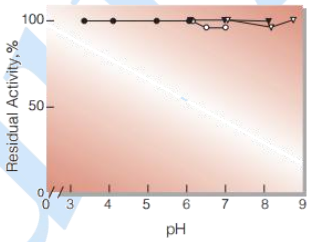


Fig.4. pH-Stability
〔25℃, 16 hr-treatment with 50mM buffer solution contg. 1mM $CaCl_2$; ●—●, acetate; ▼—▼, phosphate; ○—○, PIPES; ▽—▽, Tris-HCl.〕

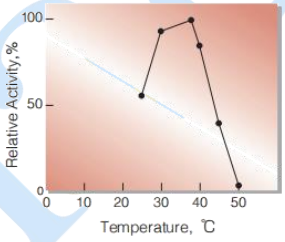


Fig.3. Temperature Activity
〔in 42mM PIPES-NaOH buffer, pH 6.5〕

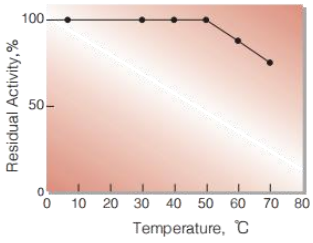


Fig.5. Thermal stability
〔30min.-treatment with 50mM PIPES-NaOH buffer, pH 6.5 contg. 1mM $CaCl_2$ enzyme concentration: 5.0 U/ml〕