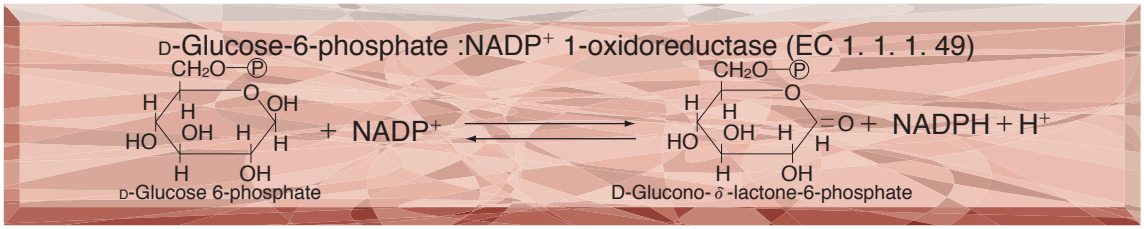
●TOYOBO ENZYMES●
(Diagnostic Reagent Grade)

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

from Leuconostoc mesenteroides



PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized	
Activity	: Grade Ⅲ 400U/mg-solid or more (NAD^+)	
Contaminants	: Creatine phosphokinase	≤1×10^{-3}%
	Phosphoglucomutase	≤1×10^{-3}%
	6-Phosphogluconate dehydrogenase	≤5×10^{-3}%
	Phosphoglucose isomerase	≤1×10^{-2}%
	Glutathione reductase	≤1×10^{-3}%
	Hexokinase	≤1×10^{-2}%
	Myokinase	≤1×10^{-2}%
	NADH oxidase	≤1×10^{-2}%
	NADPH oxidase	≤1×10^{-2}%

PROPERTIES

Stability	: Stable at −20℃ for at least one year	(Fig.1)
	Stable at 5℃ for at least 6 months (liquid form)	(Fig.3)
Molecular weight	: 104,000(two subunits of approx. 55,000) [1,2]	
Isoelectric point	: 4.6 [2]	
Michaelis constants [2]	: NAD^+ linked : 1.06×10^{-4}M (NAD^+), 5.27×10^{-5}M (G-6-P)	
	$NADP^+$ linked : 5.69×10^{-6}M ($NADP^+$), 8.1×10^{-5}M (G-6-P)	
Structure	: Neither cysteine nor cystine residues is present in the enzyme molecule [1] and essential lysine is indicated to be at active site. [3]	
Inhibitors	: Acyl-CoA,[4] ATP,[4] mental ions etc. (Table 1)	
Optimum pH	: 7.8	(Fig.4)
Optimum temperature	: 50℃	(Fig.5)
pH Stability	: pH 5.5−7.5 (30℃, 17hr)	(Fig.6)
Thermal stability	: below 37℃ (pH 8.0, 30min)	(Fig.7)
Substrate specificity	: Either NAD^+ or $NADP^+$ serves as coenzyme, the reaction velocity with NAD^+ being approximately 1.8 times greater than with NADP.[+5] D-Glucose-6-phosphate is a preferential substrate for the enzyme, although D-glucose reacts slowly.[6] Fructose-6-phosphate, fructose-1, 6-diphoshate and ribose-5-phosphate are not considered to be substrates.[7]	

APPLICATIONS

This enzyme is useful for enzymatic determation of NAD^+($NADP^+$) and G-6-P, and activities of phosphoglucose isomerase, phosphoglucomutase and hexokinase. This enzyme is also used for enzymatic determination of glucose when coupled with hexokinase (HXK-311).

ASSAY

Principle:

D-Glucose-6-phosphate(G-6-P)＋NAD^+ $\xrightarrow{\text{glucose-6-phophate dehydrogenase(G-6-PDH)}}$

D-Glucono-δ-lactone-6-phosphate＋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 7.8 : 55mM (contaning 3.3mM magnesium chloride)
B. NAD^+ solution : 60mM (Should be prepared fresh)
C. G-6-P solution : 0.1M glucose-6-phosphate (Should be prepared fresh)
D. Enzyme diluent : 5mM Tris-HCl buffer, pH 7.5, containing 0.1% of bovine serum albumin.

Procedure

1. Prepare the following reaction mixture in a cuvette (d＝1.0cm) and equilibrate at 30℃ for about 5 minutes.

2.7ml	Tris-HCl buffer, pH 7.8	(A)
0.1ml	NAD^+ solution	(B)
0.1ml	G・6・P solution	(C)

Concentration in assay mixture	
Tris-HCl buffer	50 mM
G-6-P	3.3 mM
NAD^+	2.0 mM
$MgCl_2$	3.0 mM
BSA	33 μg/ml

2. Add 0.1ml of the enzyme solution* and mix by gentle inversion.
3. Record the increase in optical density at 340nm against water for 4 to 5 minutes in a spectrophotometer thermostated at 30℃ 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 is added instead of the enzyme solution.

* Dissolve the enzyme preparation in ice-cold enzyme diluent (D) and dilute to 0.05－0.20U/ml with the same buffer, immediately before the assay.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta\text{OD/min (}\Delta\text{OD test}-\Delta\text{OD blank)}\times Vt\times df}{6.22\times 1.0\times Vs} = \Delta\text{OD/min}\times 4.82\times df$$

Weight activity (U/mg) ＝ (U/ml)×1/C

Vt : Total volume (3.0ml)
Vs : Sample volume (0.1ml)
6.22 : Millimolar extinction coefficient of NADH (㎤/micromole)
1.0 : Light path length (cm)
df : Dilution facter
C : Enzyme concentration in dissolution (c mg/ml)

REFERENCES

1) A.Ishaque,M.Mihausen and H.R.Levy; *Biochem. Biophys. Res. Comm., 59,* 894 (1974).
2) C. Olive, M.E. Geroch and H.R.Levy; *J.Biol.Chem., 246,* 2043 (1971).
3) M.Milhausen and H.R. Levy; *Eur.J.Biochem., 50,* 453 (1975).
4) E.L.Coe and L.-H.Hsu; *Biochem. Biophys. Res. Comm., 53,* 66 (1973).
5) C.Olive and H.R. Levy; *Biochem., 6,* 730 (1967).
6) R.P.Metzger, S.A. Metzger and R.L. Parsons; *Arch Biochem. Biophys., 149,* 102 (1972).
7) *Methods in Enzymology, Vol, 1,* p328 (S.P.Colowick and N.O.Kapalan,eds.), Academic Press, New York (1955).

Table 1. Effect of Various Chemicals on Glucose-6-phosphate dehydrogenase

[The enzyme dissolved in 50mM Tris-HCl buffer,pH 7.5 (5.25U/ml) was incubated with each chemcal for 1hr at 30℃.]

Chemical	Concn.(mM)	Residual activity(%)
None	—	100
Metal salt	2.0	
$AgNO_3$		86
$Ba(OAc)_2$		51
$CaCl_2$		90
$Cd(OAc)_2$		74
$CoCl_2$		80
$CuSO_4$		66
$FeCl_3$		0
$FeSO_4$		1
$HgCl_2$		84
$MgCl_2$		90
$MnCl_2$		89
$NiCl_2$		89
$Pb(OAc)_2$		3
$Zn(OAc)_2$		67
$ZnSO_4$		53
KF	2.0	93
NaF	20.0	98
NaN_3	20.0	93

Chemical	Concn.(mM)	Residual activity(%)
NEM	2.0	91
PCMB	2.0	96
MIA	2.0	14
Iodoacetamide	2.0	0
EDTA	5.0	94
$(NH_4)_2SO_4$	20.0	98
Borate	20.0	95
o-Phenanthroline	2.0	93
α,α'-Dipyridyl	2.0	95
Urea	2.0	93
Guanidine	2.0	93
Hydroxylamine	2.0	91
Na-cholate	1.0%	102
Triton X-100	1.0%	100
Brij 35	1.0%	4
SDS	0.1%	0
Tween 20	0.1%	101
Span 20	0.1%	99
DAC	0.1%	0

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

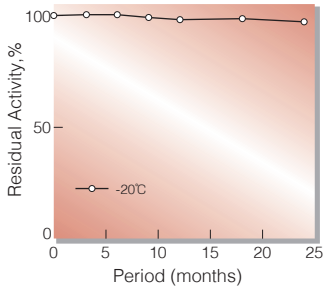


Fig.1. Stability (Powder form)

〔kept under dry conditions〕

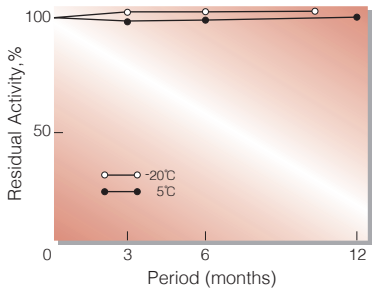


Fig.2. Stability (Powder form)

〔kept under dry conditions〕

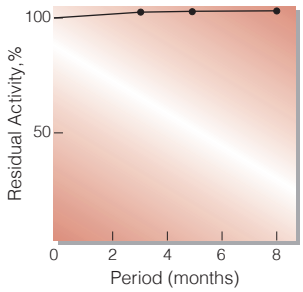


Fig.3. Stability (Liquid form at 5℃)

〔enzyme concentration:5,000U/ml composition:3.2M ammonium sulfate,pH6.0〕

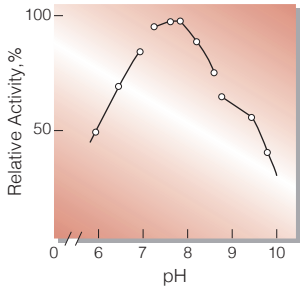


Fig.4. pH-Activity

〔30℃ in the following buffer solution: pH5.7-6.8, 15mM Veronal-CH_3COONa-HCl;pH6.8-8.5,50mM Tris-HCl; pH8.5-9.5, 50mM glycine-NaOH〕

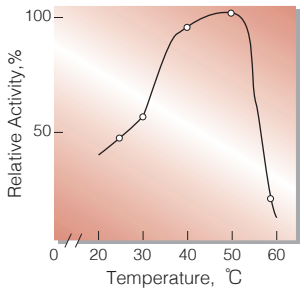


Fig.5. Temperature activity

〔in 50mM Tris-HCl buffer, pH7.8〕

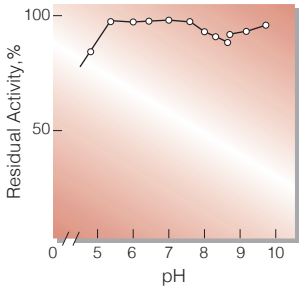


Fig.6. pH-Stability

〔30℃, 17hr-treatment with the following buffer solution: pH5.0-7.8, 30mM Veronal-CH_3COONa-HCl;pH7.5-8.5, 0.1M Tris-HCl; pH8.5-9.5,0.1M glycine-NaOH〕

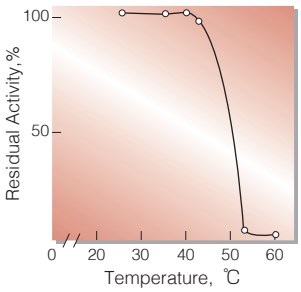


Fig.7. Thermal stability

〔30min-treatment with 5.0mM glycine-NaOH buffer, pH8.0, containing 0.1% of bovine serum albumin〕

活性測定法（Japanese）

1.原理

D-Glucose-6-phosphate(G-6-P)＋NAD^+ $\xrightarrow{\text{glucose-6-phosphate dehydrogenase (G-6-PDH)}}$ Glucono-δ-lactone-6-phosphate＋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. 55mM Tris-HCl 緩衝液,pH7.8(3.3mMの$MgCl_2$を含む)
B. 60mM NAD^+水溶液(用時調製)
C. 0.1M G-6-P(glucose-6-phosphate)水溶液(用時調製)

酵素溶液：酵素標品を予め氷冷した0.1%牛血清アルブミン(BSA)を含む5mM Tris-HCl緩衝液,pH7.5で溶解し，分析直前に同緩衝液で0.05～0.20U/mℓ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,30℃で約5分間予備加温する。

2.7mℓ	Tris-HCl 緩衝液	(A)
0.1mℓ	NAD^+水溶液	(B)
0.1mℓ	基質溶液	(C)

②酵素溶液0.1mℓを添加し,ゆるやかに混和後,水を対照に30℃に制御された分光光度計で340nmの吸光度変化を4～5分間記録し，その初期直線部分から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りに酵素希釈液(0.1%BSAを含む5mM Tris-HCl緩衝液,pH7.5)を0.1mℓ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

$$U/m\ell = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times 3.0(m\ell) \times 希釈倍率}{6.22 \times 1.0 \times 0.1(m\ell)}$$

$$= \Delta OD/min \times 4.82 \times 希釈倍率$$

U/mg ＝U/mℓ×1/C

6.22 ： NADHのミリモル分子吸光係数(cm^2/micromole)

1.0 ： 光路長(cm)

C ： 溶解時の酵素濃度(c mg/mℓ)