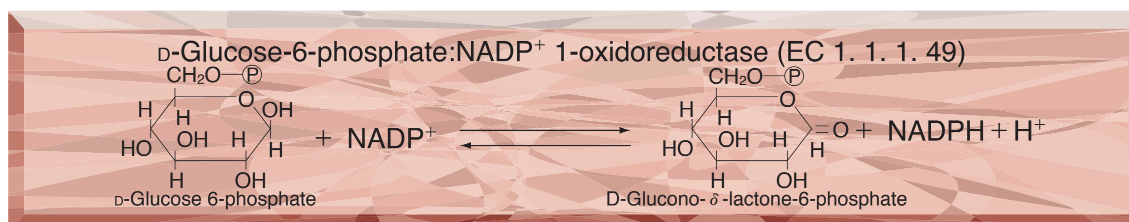
●TOYOBO ENZYMES●
(Diagnostic Reagent Grade)

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

from Microorganism



PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized
Activity	: GradeⅢ 200U/mg-solid or more
Contaminants	: Creatine phosphokinase ≤1×10⁻³%

Contaminant	Limit
Creatine phosphokinase	$\leq 1\times10^{-3}\%$
Phosphoglucomutase	$\leq 1\times10^{-3}\%$
6-Phosphogluconate dehydrogenase	$\leq 5\times10^{-3}\%$
Phosphoglucose isomerase	$\leq 1\times10^{-2}\%$
Glutathione reductase	$\leq 1\times10^{-3}\%$
Hexokinase	$\leq 1\times10^{-2}\%$
Myokinase	$\leq 1\times10^{-2}\%$
NADH oxidase	$\leq 1\times10^{-2}\%$
NADPH oxidase	$\leq 1\times10^{-2}\%$

PROPERTIES

Stability	: Stable −20℃ for at least one year	(Fig.1)
Molecular weight	: approx. 140,000 (by gel filtration)	
Michaelis constants	: NAD^+ linked : 2.4×10^{-4}M (NAD^+), 4.7×10^{-4}M (G-6-P)	
	$NADP^+$ linked : 7.4×10^{-6}M ($NADP^+$), 3.2×10^{-4}M (G-6-P)	
Inhibitors	: Metal ions, iodoacetamimide, SDS etc.	
Optimum pH	: 7.8	(Fig.2)
Optimum temperature	: 50−55℃	(Fig.3)
pH Stability	: pH 5.0−11.0 (25℃, 22hr)	(Fig.4)
Thermal stability	: below 50℃ (pH 7.8, 30min)	(Fig.5)
Substrate specificity	: (Table 1)	
Effect of various chemicals	: (Table 2)	

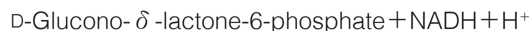
APPLICATIONS

This enzyme is useful for enzymatic determination of NAD^+($NADP^+$) and G-6-P, and activities of phosphoglucose isomerase, phosphoglucomutase and hexokinase. The enzyme is also used for enzymatic determination of glucose and creatine phosphokinase activity 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 (containing 3.3mM magnesium chloride)
B. NAD^+ solution	: 60mM (Should be prepared fresh)
C. G-6-P solution	: 0.1M D-Glucose-6-phosphate (should be prepared fresh)
D. Enzyme diluent	: 5mM Tris-HCl buffer, pH 7.5, containing 0.1% 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, pH 7.8	(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 of 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 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) 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 :

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

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 under the assay condition (cm^2/micromole)
1.0	: Light path length (cm)
df	: Dilution facter
C	: Enzyme concentration in dissolution

Table 1. Substrate specificity of Glucose-6-phosphate dehydrogenase
[3.3mM of substrate, 50mM Tris-HCl buffer, pH 7.8]

Substrate	Relative activity(%)
Glucose-6-phosphate	100
Fructose-6-phosphate	0
Glucose-1-phosphate	0
Gluconate-6-phosphate	0

Table 2. Effect of Various Chemicals on Glucose-6-phosphate dehydrogenase
[The enzyme dissolved in 50mM Tris-HCl buffer, pH 7.5 (10U/ml) was incubated with each chemical for 1hr at 30℃.]

Chemical	Concn.(mM)	Residual activity(%)
None	—	100
Metal salt	2.0	
$AgNO_3$		74
$Ba(OAc)_2$		100
$CaCl_2$		95
$Ca(OAc)_2$		84
$CoCl_2$		94
$CuSO_4$		84
$FeCl_3$		0
$FeSO_4$		0
$HgCl_2$		87
$MgCl_2$		100
$MnCl_2$		97
$NiCl_2$		94
$Pb(OAc)_2$		31
$Zn(OAc)_2$		63
$ZnSO_4$		76
KF	2.0	103
NaF	20.0	99
NaN_3	20.0	102

Chemical	Concn.(mM)	Residual activity(%)
NEM	2.0	94
PCMB	2.0	103
MIA	2.0	99
Iodoacetamide	2.0	0
EDTA	5.0	102
$(NH_4)_2SO_4$	20.0	99
Borate	20.0	98
o-Phenanthroline	2.0	100
α,α'-Dipyridyl	2.0	102
Urea	2.0	99
Guanidine	2.0	100
Hydroxylamine	2.0	100
Na-cholate	1.0%	106
Triton X-100	1.0%	104
Brij 35	1.0%	12
SDS	0.1%	1
Tween 20	0.1%	102
Span 20	0.1%	101
DAC	0.1%	1

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

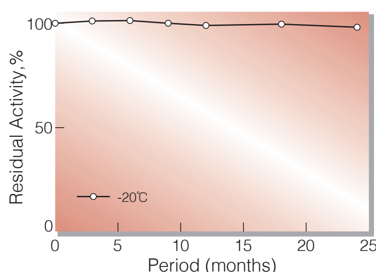


Fig.1. Stability (Powder form)
[kept under dry conditions]

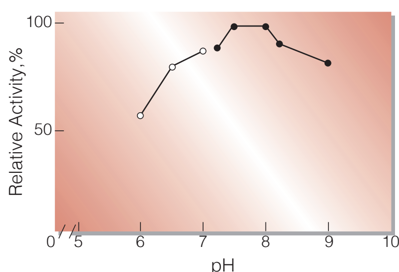


Fig.2. pH-Activity
[30℃ in the following buffer solution: pH6.0-7.0, 50mM PIPES pH7.2-9.0, 50mM Tris-HCl]

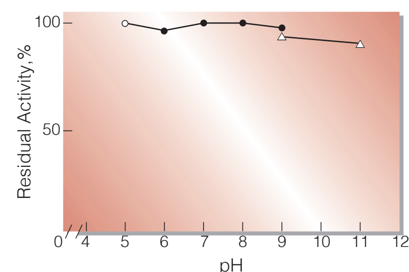


Fig.4. pH-Stability
[25℃, 22hr-treatment with the following 0.1M buffer solution: pH5.0-6.0,Acetate; pH6.0-9.0,K-phosphate; pH9.0-11.0,Glycine-NaOH]

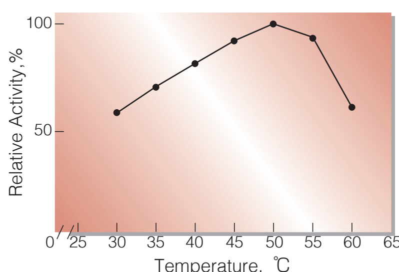


Fig.3. Temperature activity
[in 50mM Tris-HCl buffer, pH7.8]

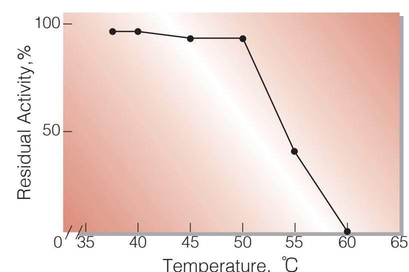


Fig.5. Thermal stability
[30min-treatment with 5.0mM Tris-HCl buffer, pH7.8, containing 0.1% of bovine serum albumin]

ENZYMES Diagnostic Reagent Grade

活性測定法（Japanese）

1.原理

生成したNADHの生成量を340nmにおける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.　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。

5.計算式

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

$$= \Delta \mathrm{OD/min} \times 4.82 \times \text{希釈倍率}$$

U/mg ＝U/mℓ×1/C

6.22　：上記測定条件における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 mg/mℓ)