

GLUCOSE DEHYDROGENASE [FAD-GDH II]

from Microorganism

(FAD dependent glucose dehydrogenase, EC 1.1.5.9)



Preparation and Specification

Appearance : Light yellow to yellow lyophilizate

Specific activity : More than 400 U/mg solid

Properties

Molecular weight : 66 kDa (SDS-PAGE, as deglycosylated form)

Michaelis constant : 1.3×10^{-2} M (glucose)

Optimum pH : See Figure 1

pH stability : See Figure 2

Optimum temperature : See Figure 3

Thermal stability : See Figure 4

Substrate specificity : See Table 1

Light stability : See Table 2

Stability (solid form) : See Table 3

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of glucose.

Table 1. Substrate specificity

Substrate	Relative activity (%)
Glucose	100
Galactose	0.6
Xylose	2.4
Maltose	0.4
Mannose	1.0
2-deoxy-glucose	31.8

Fig.1 Optimum pH

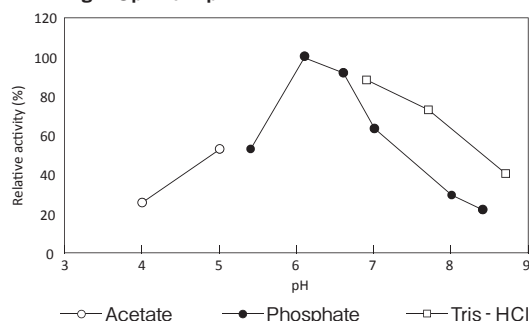


Table 2. Light stability

Form of FAD-GDH	Light source	Residual activity (%)
Powder	LED	93.2
	Fluorescent lamp	93.7
Solution (1mg/ml, water)	LED	95.7
	Fluorescent lamp	96.4

Radiation for 24 hours

Fig.2 pH Stability

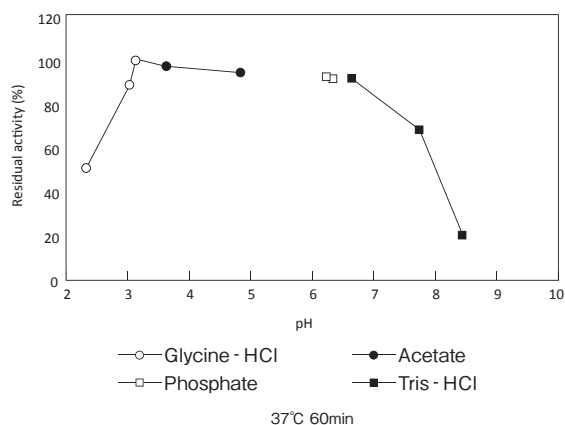


Fig.3 Optimum Temperature

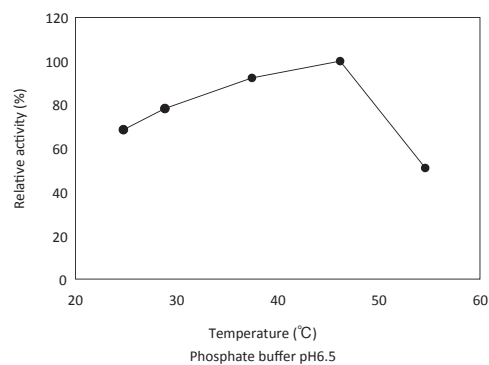


Fig.4 Thermal stability

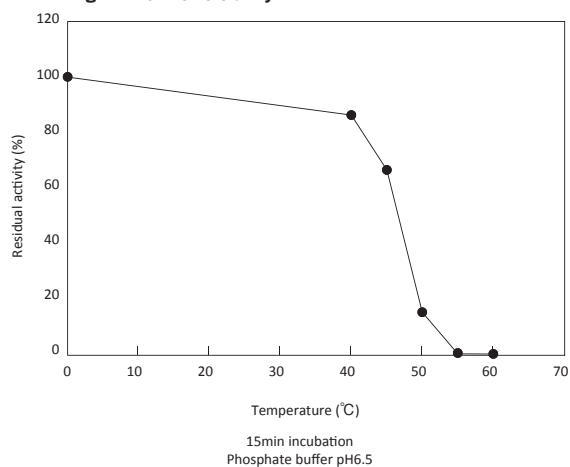


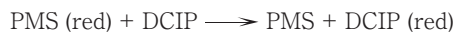
Table 3. Stability (solid form)

Storage condition	Residual activity (%)
-20°C 1046 days	93.2

Assay

Principle

The assay is based on the decrease in absorbance at 600 nm as the reduction of 2,6-dichloroindophenol in the following reactions:



PMS : Phenazine methosulfate

DCIP : 2,6-Dichloroindophenol

Unit definition

One unit is defined as the amount of enzyme which converts 1 μ mole of D-Glucose to D-Glucono-1,5-lactone per minute at 37 °C under the conditions specified in the assay procedure.

Reagents

1. Reaction mixture
0.1 M $\text{NaH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ buffer pH 6.5 containing 0.222 M D-Glucose and 0.0833% Triton X-100
2. 20 mM PMS solution
3. 2.0 mM DCIP solution

4. Enzyme dilution buffer

50 mM $\text{NaH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ buffer pH 6.5 containing 0.1 % BSA and 0.1 % Triton X-100

5. Reagents

Triton X-100 : The Dow Chemical Company

BSA : Millipore Fraction V pH5.2 #81053

D-Glucose : FUJIFILM Wako Pure Chemical Corporation
049-31165

PMS (Phenazine methosulfate) :

Tokyo Chemical Industry # P0083

DCIP (2,6-Dichloroindophenol) : Sigma-Aldrich

119814

Enzyme solution

Accurately weigh about 10 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

Procedure

1. Pipette accurately 1.8 ml of reaction mixture and 20 μ l of enzyme solution into a small test tube. Add 100 μ l of 20 mM PMS solution, mix and preincubate at 37 °C.
* In the case of a blank test, add 20 μ l of enzyme dilution buffer at this time.

- After 3 min, add 100 μ l of 2.0 mM DCIP solution and mix to start the reaction at 37 $^{\circ}$ C.
- After starting the reaction, measure the rate of increase per minute in absorbance at 600 nm. The rate must be measured within the linear portion of the absorbance curve.

Absorbance sample : As/min
blank : Ab/min

$$\Delta A/\text{min} = (As/\text{min} - Ab/\text{min}) \leq 0.1 \text{ Abs}/\text{min}$$

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\Delta A/\text{min}}{16.3} \times \frac{2.02}{0.02} \times \frac{1}{X}$$

- 16.3 : millimolar extinction coefficient of DCIP at 600 nm ($\text{cm}^2/\mu\text{mole}$)
2.02 : final volume (ml)
0.02 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at -20 $^{\circ}$ C in the presence of a desiccant is recommended.

References

- Matsushita, K. et al. (1980) Agric. Biol. Chem. 44, 1505-1512.
- Shinagawa, E. et al. (1990) J. Biochem. 107, 863-867.

FAD-GDH II 活性測定法 (Japanese)

I. 試薬液

- 反応試薬混合液
0.222 M グルコース及び 0.0833 % トリトン X-100
含む 0.1 M リン酸緩衝液 pH 6.5
- 20 mM PMS 溶液
- 2.0 mM DCIP 溶液
- 酵素溶解希釈用液
0.1 % BSA 及び 0.1 % トリトン X-100 を含む 50
mM リン酸緩衝液 pH 6.5
- 試薬
トリトン X-100: Dow Chemical 製
BSA (Bovine serum albumin) Fra.V, pH5.2:
Millipore 製 # 81053
グルコース:
富士フイルム和光純薬製 特級 # 049-31165
PMS (フェナジンメチルスルファート):
東京化成製 # P0083
DCIP (2,6-ジクロロインドフェノールナトリウム塩):
シグマ製 # 119814

II. 酵素試料液

検品約 10 mg を精密に量り、酵素溶解希釈用液に溶解して全容 20 ml とする。その液を酵素溶解希釈用液で適宜希釈する。

III. 測定操作法

- 小試験管に反応試薬混合液 1.8 ml 及び酵素試料液 20 μ l を正確に分注し、20 mM PMS 溶液 100 μ l を加えて混和し、37 $^{\circ}$ C で予備加温する。
※盲検は酵素試料液の代わりに酵素溶解希釈用液 20 μ l を加える。
- 3 分経過後、2.0 mM DCIP 溶液 100 μ l を加えて混和し、37 $^{\circ}$ C で反応を開始する。
- 反応開始後、600 nm における吸光度を測定して直線的に反応している 1 分間当たりの吸光度変化を求める。求められた吸光度変化を試料液については As/min、盲検液については Ab/min とする。
$$\Delta A/\text{min} = (As/\text{min} - Ab/\text{min}) \leq 0.1 \text{ Abs}/\text{min}$$

IV. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A/\text{min}}{16.3} \times \frac{2.02}{0.02} \times \frac{1}{X}$$

- 16.3 : DCIP の 600nm におけるミリモル分子吸光係数 ($\text{cm}^2/\mu\text{mole}$)
2.02 : 反応総液量 (ml)
0.02 : 反応に供した酵素試料液量 (ml)
X : 酵素試料液中の検品濃度 (mg/ml)