GLUCOSE DEHYDROGENASE [FAD-GDH Ⅱ]

from Microorganism

(FAD dependent glucose dehydrogenase, EC 1.1.5.9)

D-Glucose + acceptor ⟶ D-glucono-1,5-lactone + reduced acceptor

G

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
Stability (solid form) : See Figure 5
Substrate specificity : See Table 1
Light stability : See Table 2

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of glucose.

Table 1. Substrate specificity

Substrate	FAD-GDH II	FAD-GDH from *Aspergillus sp.*
Glucose	100	100
Galactose	0.6	0.3
Xylose	2.4	13.5
Maltose	0.4	0
Mannose	1.0	1.3
2-deoxy-glucose	31.8	29.2

Fig.1 Optimum pH

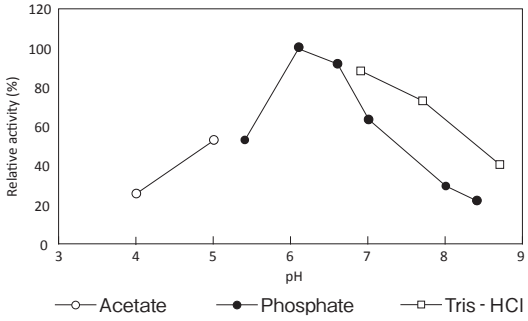


Table 2. Light stability

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

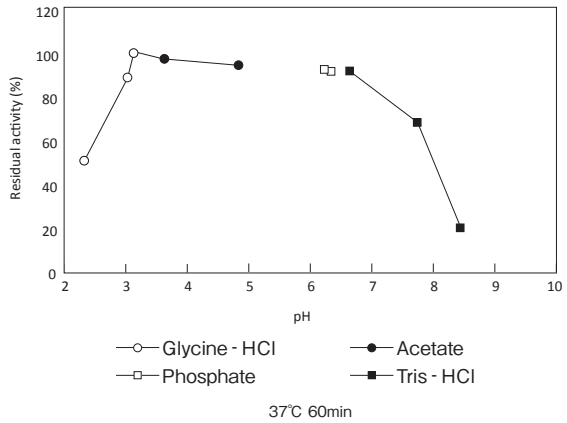


Fig.3 Optimum Temperature

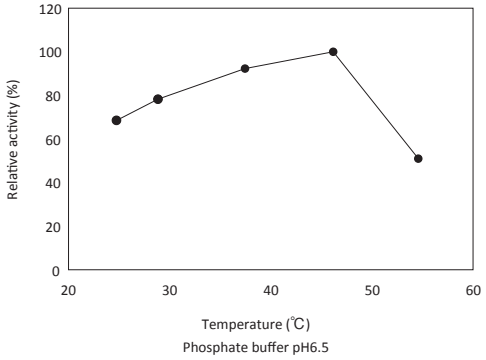


Fig.4 Thermal stability

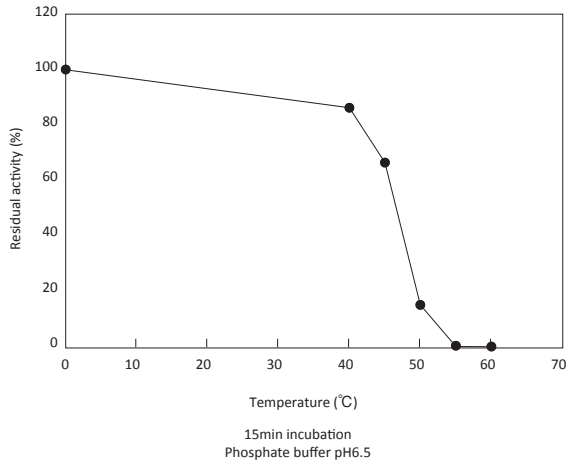
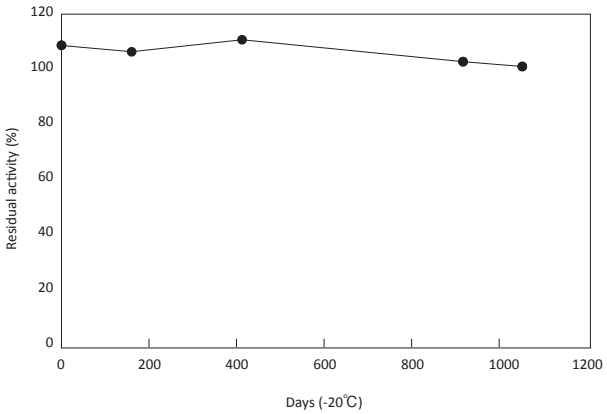


Fig.5 Stability (solid form)



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:

$$\text{D-Glucose} + \text{PMS} \xrightarrow{\text{FAD-GDH II}} \text{D-Glucono-1,5-lactone} + \text{PMS (red)}$$

$$\text{PMS (red)} + \text{DCIP} \longrightarrow \text{PMS} + \text{DCIP (red)}$$

PMS : Phenazine methosulfate
DCIP : 2,6-Dichloroindophenol

■ Unit definition

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

■ Reagents

1. Reaction mixture
   0.1 M NaH_2PO_4–Na_2HPO_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 NaH_2PO_4–Na_2HPO_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 μl of enzyme solution into a small test tube. Add 100 μl of 20 mM PMS solution, mix and preincubate at 37 ℃.
   ※ In the case of a blank test, add 20 μl of enzyme dilution buffer at this time.

2. After 3 min, add 100 μl of 2.0 mM DCIP solution and mix to start the reaction at 37 ℃.
3. 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
△ A/min = (As/min − Ab/min) ≦ 0.1Abs/min

■ **Calculation**

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

16.3 : millimolar extinction coefficient of DCIP at 600 nm (cm^2/ μmole)
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 ℃ in the presence of a desiccant is recommended.

References

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

G

FAD-GDH Ⅱ活性測定法 (Japanese)

Ⅰ. 試薬液

1. 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
2. 20 mM PMS 溶液
3. 2.0 mM DCIP 溶液
4. 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
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

1. 小試験管に反応試薬混合液 1.8 ml 及び酵素試料液 20 μl を正確に分注し、20 mM PMS 溶液 100 μl を加えて混和し、37 ℃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 μl を加える。
2. 3 分経過後、2.0 mM DCIP 溶液 100 μl を加えて混和し、37 ℃で反応を開始する。
3. 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。
   Δ A/min = (As/min − Ab/min) ≦ 0.1 Abs/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 (cm^2/μmole)
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)