Recombinant Glucose Dehydrogenase (GDH-FAD)

G1493019

Storage temperature: -20℃. Avoid freeze/thaw cycle. Store in the dark.

Introduction:

Recombinant Glucose Dehydrogenase (GDH-FAD) is an FAD-dependent glucose dehydrogenase with low reactivity toward maltose and xylose. It has high stability and maintains its reactivity even at low temperatures.

D-Glucose + acceptor → D-Glucono-1,5-lactone + reduced acceptor

Specifications

Appearance: Yellow lyophilized powder

Activity: ≥700 U/mg lyophilized powder

Contaminants:

NAD Glucose Dehydrogenase < 1.0×10^{-2} %

β-Glucosidase < 1.0×10^{-2} %

Hexokinase < 1.0×10^{-2} %

α-Glucosidase < 1.0×10^{-2} %

Characteristics

Molecular weight: Approximately 85 kDa (determined by SDS-PAGE)

Structure: Monomer, 1 mol FAD per mol glycoprotein

Michaelis constant: 2.2×10^{-2} m (D-glucose)

Optimal pH: 7.0-7.5 Fig. 1

pH stability: 4.5-8.0 Fig. 2

Optimal temperature: 40-50°C Fig. 3

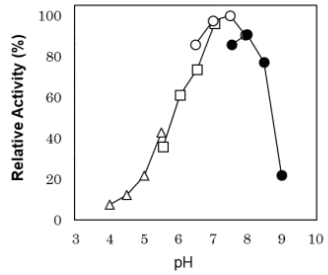
Thermal stability (liquid form): Below 50°C Fig. 4

Thermal stability (powder form): stable at 30°C for at least 20 days Fig. 5

Specificity: Table 1

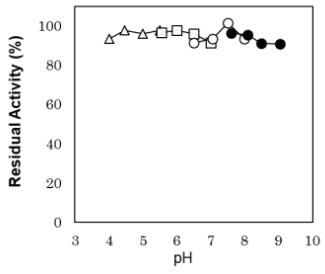
Inhibitor: Mn^{2+}, Ag^{+}

Fig.1 pH Optimum



△: 0.1 M acetate buffer
□: 0.1 M MES-NaOH buffer
○: 0.1 M phosphate buffer
●: 0.1 M Tris-HCl buffer

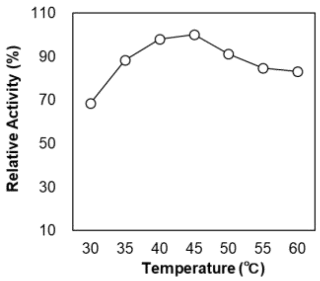
Fig.2 pH Stability



Treatment :25℃, 16h

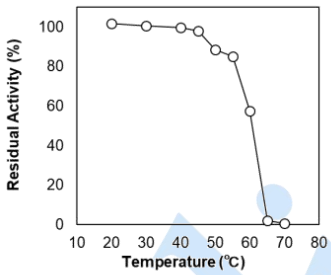
△: 0.1 M acetate buffer
□: 0.1 M MES-NaOH buffer
○: 0.1 M phosphate buffer
●: 0.1 M Tris-HCl buffer

Fig.3 Optimum temperature



Buffer :0.1 M phosphate buffer, pH 7.0

Fig.4 Thermal stability (liquid form)



Treatment :10 mM phosphate buffer, pH 6.0, containing 0.1% BSA, 15 min

Fig.5 Thermal stability (powder form) at 30℃

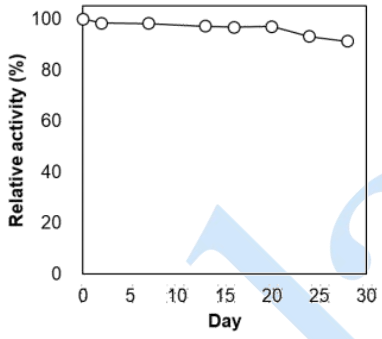


Table 1 Substrate specificity

Substrate	Relative activity (%)
D-Glucose	100
Maltose	<0.01
D-Xylose	8.7
D-Galactose	0.7
Sucrose	0.1
D-Mannose	3.9
2-Deoxy-D-glucose	52.2

ASSAY PROCEDURE

Principle

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

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

The disappearance in the blue color of DCIP due to reduction is measured spectrophotometrically at 600 nm.

Reagents

A. D-Glucose solution, 2 M: 72 g D-glucose/200 ml distilled water.

B. Potassium phosphate buffer, 0.1 M, pH 7.0: Mix 0.1 m KH_2PO_4 solution and 0.1 M KH_2PO_4 solution to make a pH 7.0 solution.

C. 2,6-Dichloroindophenol (DCIP) solution, 1.8 mM: 58.7 mg DCIP/100 ml distilled water.

D. Phenazine methosulfate (PMS) solution, 30 mM: 91.9 mg PMS/10 ml distilled water.

E. Enzyme dilution buffer: 10 mM potassium phosphate buffer, pH 6.0, containing 0.1% bovine serum albumin (BSA).

Sample: dissolve the lyophilized enzyme to final concentration about 0.4 μg/mL with enzyme dilution buffer (Reagent E) immediately before measurement.

Procedure

1. Pipette the following reagents into a cuvette (light path: 1 cm):

600 μL	D-glucose solution	(Reagent A)
2050 μL	potassium phosphate buffer, pH 7.0	(Reagent B)
150 μL	DCIP solution	(Reagent C)

2. Equilibrate at 37°C for about 3 minutes.
3. Add 0.1 ml PMS solution (Reagent D) and mix.
4. Add 0.1 ml sample and mix.
5. Record the decrease of absorbance at 600 nm against water for 1 min. (30–90 sec) in a spectrophotometer thermostated at 37°C, and calculate the ΔA per min using the linear portion of the curve (ΔA_S). The blank solution is prepared by adding Enzyme dilution buffer (Reagent E) instead of sample (ΔA_0).

Calculation

The activity can be calculated by the following formula:

$$\text{Volume activity (U/mL)} = \frac{(\Delta A_S - \Delta A_0) \times 3\ (\text{mL}) \times df}{20.4 \times 1.0 \times 0.1\ (\text{mL})} = (\Delta A_S - \Delta A_0) \times 1.47 \times df$$

20.4: Millimolar extinction coefficient of DCIP under the assay condition (cm²/μmol);

1.0: Light pass length (cm);

df: Dilution factor.