Recombinant Glucose Dehydrogenase (GDH-FAD)

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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: ≥475 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 90 kDa (determined by SDS-PAGE)

Structure: Monomer, 1 mol FAD per mol glycoprotein

Michaelis constant: 9.5×10^{-2} m (D-glucose)

Optimal pH: 7.0 - 7.5 (Fig. 1)

pH stability: 2.5 - 7.5 (Fig. 2)

Optimal temperature: 40 - 50°C (Fig. 3)

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

Inhibitor: Ag^{+}

Specificity: D-Glucose (100), Maltose (<1), D-Xylose (<1), D-Galactose (<1)

Assay Method

Principle

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

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

The decrease 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 solution of pH 7.0.

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: Immediately before the measurement, dissolve the lyophilized enzyme with the enzyme dilution buffer (Reagent E) to give a final concentration of approximately 0.4 μg/ml.

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. In a spectrophotometer thermostated at 37°C, measure the decrease in absorbance at 600 nm for 1 minute (30 - 90 seconds) using water as a reference, and calculate ΔA per minute (ΔA_S) using the linear portion of the curve. A blank solution is prepared by replacing the sample with the enzyme dilution buffer (Reagent E) (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 conditions (cm²/μmol)

1.0: Light pass length (cm)

df: Dilution factor

Applications

This enzyme can be used for the determination of D-glucose in clinical analysis and self-monitoring by glucose meters.

Experimental Data

Fig. 1 pH Optimum

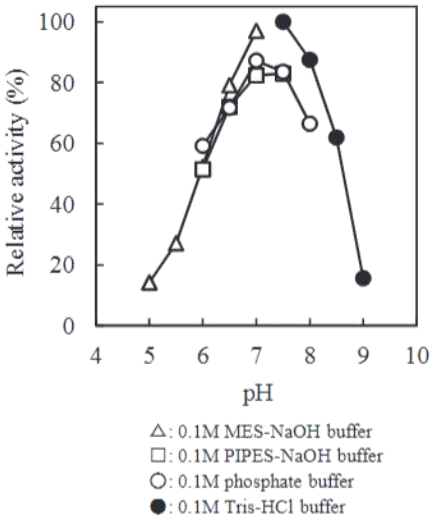

Fig. 2 pH Stability

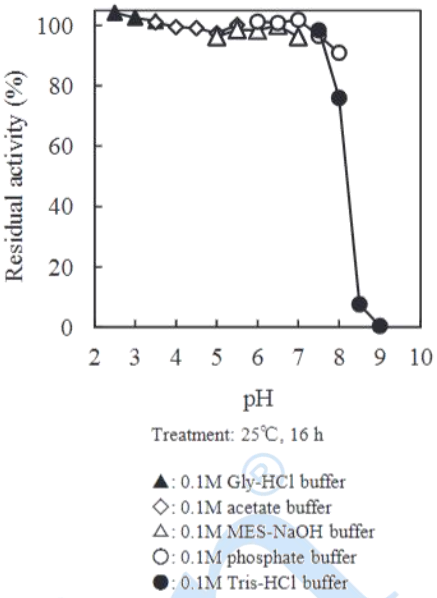

Fig. 3 Optimum temperature

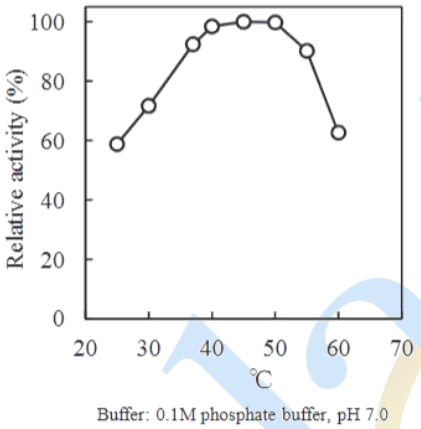

Fig. 4 Thermal stability