Glucose Dehydrogenase (FAD)

(EC 1.1.5.9, from Microorganism)

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

PREPARATION and SPECIFICATION

Appearance	Yellow amorphous powder, lyophilized
Protein purity	≥90% (from SDS-PAGE)
Activity	≥80 U/mg solid
Glucose dehydrogenase (NAD)	≤0.02%
Hexokinase	≤0.02%
α-glucosidase	≤0.02%

PROPERTIES

EC number	1.1.5.9	
Molecular weight	66 kDa (SDS-PAGE)	
Isoelectric point	5.04	
Michaelis constant	7.45×10^{-2} M (D-glucose)	
Inhibitor	Ag^{+}, Cu^{2+}, SDS	
Optimum pH	7.0	Fig. 1
Optimum temperature	50-55 °C	Fig. 2
pH stability	pH 5.0 - 9.0 (25 °C, 16 h)	Fig. 3
Thermal stability	Stable at 50 °C and blow (pH 7.0, 30 min)	Fig. 4
Storage stability	At least one year at -20 °C	Fig. 5

APPLICATIONS

This enzyme is useful for clinical analysis of D-glucose determination in blood glucose meter.

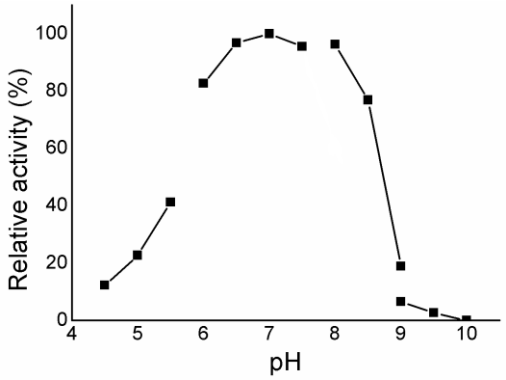

Fig. 1 Optimum pH

Buffer solution: pH 4.5-5.5, Acetate; pH 6.0-7.5, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10, Glycine-NaOH.
Enzyme concentration: 1mg/ml.

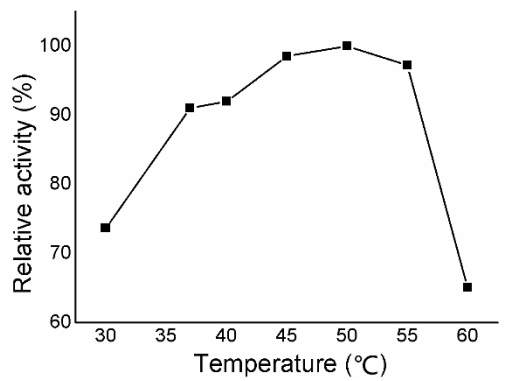

Fig. 2 Optimum temperature

Reaction in 100 mM K-phosphate buffer pH 7.0.
Enzyme concentration: 1 mg/mL.

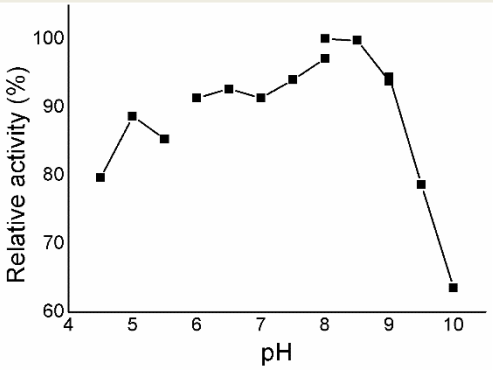

Fig. 3 pH Stability

25 °C, 16 h-treatment with 50 mM buffer solution: pH 4.5-5.5, Acetate; pH 6.0-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10, Glycine-NaOH.
Enzyme concentration: 1 mg/ml.

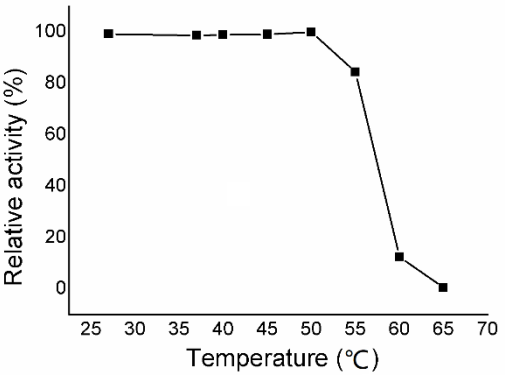

Fig. 4 Thermal stability

30 min-treatment with 100 mM K-phosphate buffer, pH 7.0.
Enzyme concentration: 1 mg/mL.

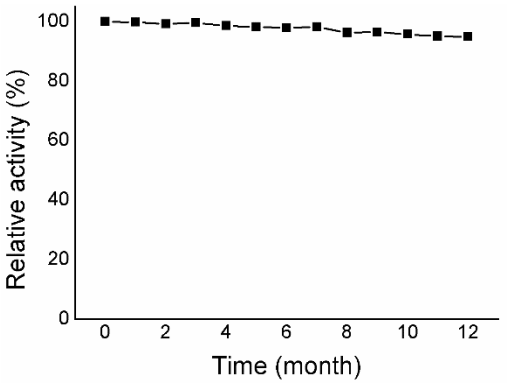

Fig.5 Storage stability (-20 °C)

ASSAY

Principle

Glucose Dehydrogenase

D-Glucose + PMS ⟶ D-Glucono-1,5-lactone +PMS (red)

PMS (red) +DCIP ⟶ PMS + DCIP (red)

The assay is based on the increase in absorbance at 600 nm as the formation of DCIP in the forward reactions.

Unit definition

One unit (U) is defined as the amount of enzyme which generates 1 μmol of DCIP per minute at 37 °C under the conditions described below.

Reagents preparation

Reagent I: 0.1 M potassium phosphate buffer, pH 7.0.

Reagent II: 2 M D-glucose solution.

Reagent III: 1.8 mM DCIP solution (be prepared when using).

Reagent IV: 30 mM PMS solution.

Enzyme dilution buffer: 0.1 M potassium phosphate buffer pH 6.0, contains 0.1% BSA.

Sample: The enzyme was diluted to 0.1-0.9 U/mL with enzyme dilution buffer.

Procedure

1. Add 2.05 mL reagent I, 0.6 mL reagent II and 0.15 mL reagent III to the 3 mL cuvette;

2. Preincubate the mixture at 37 °C for 5 min;

3. Add 0.1 mL reagent IV to the 3mL cuvette and mix;

4. Add 0.1 mL enzyme solution to the 3 mL cuvette and mix;

5. Record the ΔA_s at 600 nm in 1 minute in a spectrophotometer thermostated at 37 °C;

6. Replace the enzyme solution with enzyme dilution buffer, and record the change of blank absorbance (ΔA_b) in the same steps;

$\Delta A = \Delta A_s - \Delta A_b$

Calculation

$$\boldsymbol{Volume\quad activity}(\boldsymbol{U/ml}) = \frac{\boldsymbol{\Delta A} \times \mathbf{Vt} \times \mathbf{df}}{\mathbf{20.4} \times \boldsymbol{Vs} \times \mathbf{1.0}} = \boldsymbol{\Delta A} \times \mathbf{1.47} \times \mathbf{df}$$

$$\boldsymbol{W}\mathbf{eight}\quad \mathbf{activity}(\boldsymbol{U/mg}) = \boldsymbol{Volume\ activity} \times \mathbf{1}/\boldsymbol{C}$$

Vt: Total volume (3 mL);

Vs: Enzyme volume (0.1 mL);

1.0: Light path length (cm);

df: Dilution factor;

C: Enzyme concentration (mg/mL);

20.4: Millimolar extinction coefficient of quinoneimine dye under 600 nm (cm^2/μmol).

References

Satake, R. et al., J. Biosci. Bioeng., 120, 498–503 (2015).