Glucose Dehydrogenase (FADGDH-AA)

from recombinant *Aspergillus sojae*

D-Glucose : acceptor 1-oxidoreductase, EC 1.1.5.9

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

SPECIFICATION

Appearance	Yellow lyophilizate	
Activity	≧500 U/mg lyophilizate	
Contaminant	NAD Glucose Dehydrogenase	$<1.0\times10^{-2}$%
	Hexokinase	$<1.0\times10^{-2}$%
	α-Glucosidase	$<1.0\times10^{-2}$%
	β-Glucosidase	$<1.0\times10^{-2}$%
Storage	below −20°C	

PROPERTIES

Molecular weight	*ca*. 90 kDa (SDS-PAGE)	
Structure	monomer, one mole of FAD per mole of enzyme glycoprotein	
Michaelis constant	9.5×10^{-2} M (D-Glucose)	
pH Optimum	7.0–7.5	(Fig. 1)
pH Stability	2.5–7.5	(Fig. 2)
Optimum temperature	40–50°C	(Fig. 3)
Thermal stability	below 50°C	(Fig. 4)
Inhibitors	Ag^{+}	
Specificity	D-glucose (100), Maltose (<1),	
	D-xylose (<1), D-galactose (<1)	

ASSAY PROCEDURE

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 disappearance of the blue color of DCIP by the reduction is measured spectrophotometrically at 600 nm.

Definition of unit

One unit (U) causes the reduction of one micromole of DCIP per minute under the conditions described below.

Reagents

A. D-Glucose solution, 2 M: 72 g of D-glucose/200 ml of distilled water.
B. Potassium phosphate buffer, 0.1 M; pH 7.0: mix 0.1 M KH_2PO_4 solution and 0.1 M K_2HPO_4 solution to make a pH 7.0 solution.
C. 2,6-Dichloroindophenol (DCIP) solution, 1.8 mM: 58.7 mg of DCIP/100 ml of distilled water.
D. 5-Methylphenazinium methyl sulfate (PMS) solution, 30 mM: 91.9 mg of PMS/10 ml of 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 a volume activity of 0.1–0.9 U/ml with ice-cold 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 D)

2. Equilibrate at 37°C for about 3 min.
3. Add 0.1 ml of PMS solution (Reagent D) and mix.
4. Add 0.1 ml of 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

Activity can be calculated by using 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 ($cm^2/\mu mol$)
1.0 : Light pass length (cm)
df : Dilution factor

APPLICATIONS

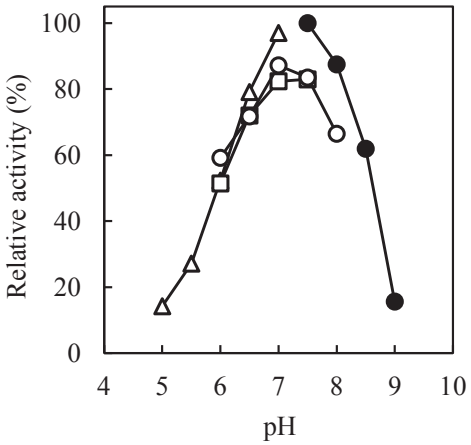
The enzyme is useful for the determination of D-glucose in clinical analysis and self-monitoring blood glucose meters.

REFERENCES

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

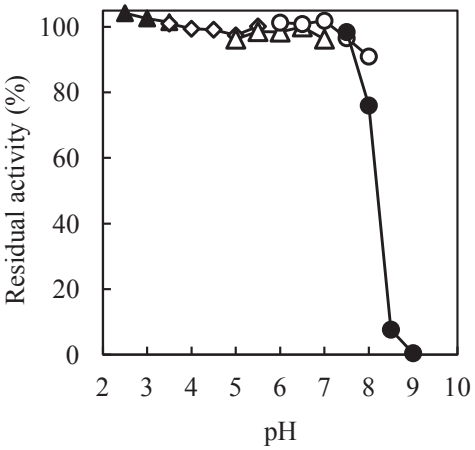
EXPERIMENTAL DATA

Fig. 1 pH Optimum



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

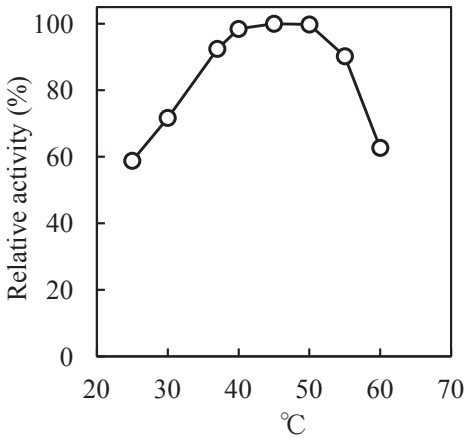
Fig. 2 pH Stability



Treatment: 25℃, 16 h

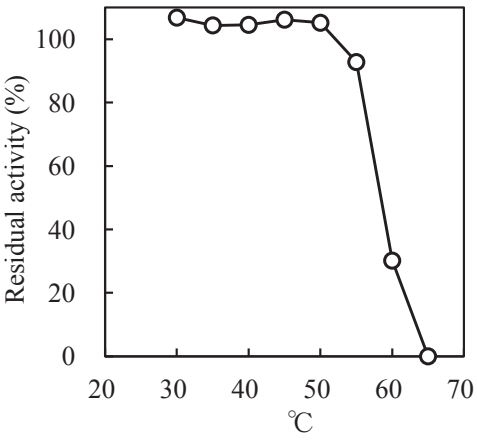
▲: 0.1M Gly-HCl buffer
◇: 0.1M acetate buffer
△: 0.1M MES-NaOH buffer
○: 0.1M phosphate buffer
●: 0.1M Tris-HCl buffer

Fig. 3 Optimum temperature



Buffer: 0.1M phosphate buffer, pH 7.0

Fig. 4 Thermal stability



Treatment: 50 mM phosphate buffer, pH 6.0, 15 min