FAD-dependent Glucose Dehydrogenase with High Stability

Kikkoman Biochemifa Company introduces a recombinant FAD-dependent Glucose Dehydrogenase (FADGDH-AD) with high stability.

PRODUCT

Product name	Glucose Dehydrogenase (FADGDH-AD)
CD	60102
Origin	Recombinant *Aspergillus sojae*
Systematic name	D-Glucose : acceptor 1-oxidoreductase
EC	1.1.5.9

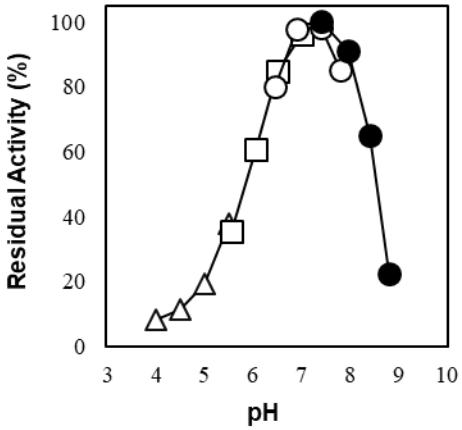
SPECIFICATION

Appearance	Yellow to brown lyophilizate	
Activity	≧700 U/mg lyophilizate	
Contaminants	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 protected from light	

PROPERTIES

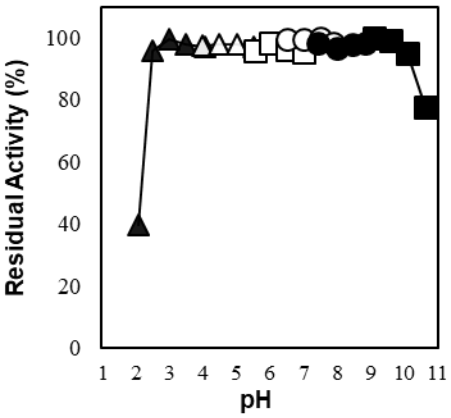
Molecular weight	ca. 90 kDa (SDS-PAGE)	
Structure	monomer, one mole of FAD per mole of enzyme glycoprotein	
Michaelis constant	6.4×10^{-2} M (D-Glucose)	
pH Optimum	7.0 - 7.5	Fig. 1
pH Stability	2.5 - 9.5	Fig. 2
Optimum temperature	45°C	Fig. 3
Thermal stability (liquid form)	below 60°C	Fig. 4
Thermal stability (powder form)	stable at 30°C for at least one month	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

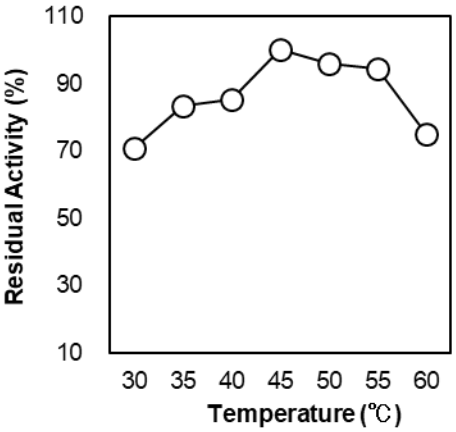
Fig.2 pH Stability



Treatment :25℃, 16h

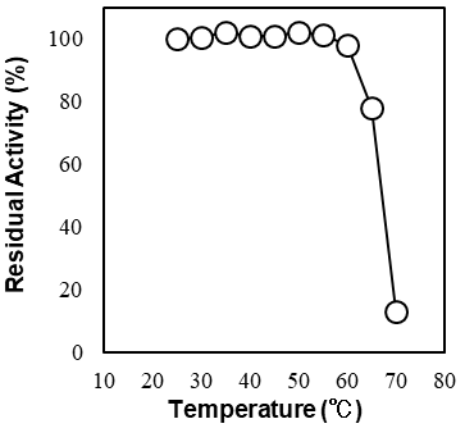
▲: 0.1 M Glycine-HCl buffer
△: 0.1 M acetate buffer
□: 0.1 M MES-NaOH buffer
○: 0.1 M phosphate buffer
●: 0.1 M Tris-HCl buffer

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, 15min

Fig.5 Thermal stability (powder form)

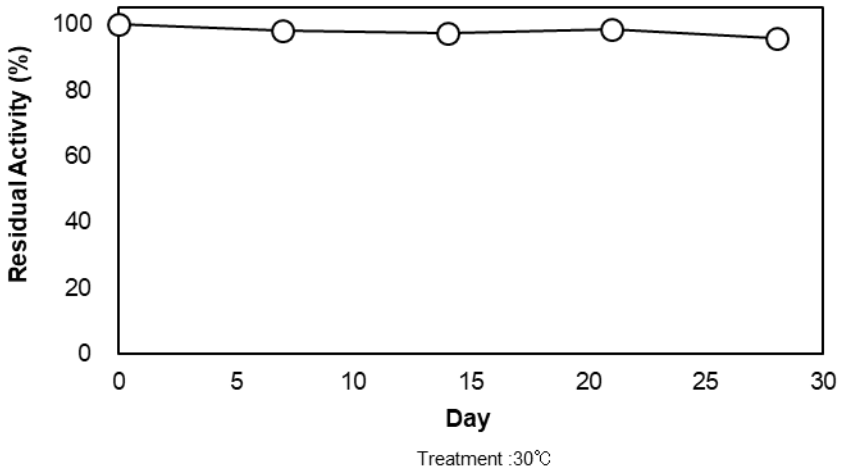


Table 1 Substrate specificity

Substrate	Relative activity (%)
D-Glucose	100
Maltose	0.2
D-Xylose	0.9
D-Galactose	0.8
Sucrose	<0.1
D-Mannose	0.4
2-Deoxy-D-glucose	23.5

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 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: 1cm).

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 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/μmol)

1.0: Light pass length (cm)

df: Dilution factor

APPLICATIONS

The enzyme is useful for the determination of D-Glucose in clinical analysis and continuous glucose monitoring (CGM) meter.

REFERENCES

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