Glucose Dehydrogenase“Amano”8DC

(D-Glucose: acceptor 1-oxidoreductase, EC 1.1.5.9)

Glucose Dehydrogenase“Amano”8DC is a FAD-dependent glucose dehydrogenase preparation manufactured by a unique fermentation process using a selected strain of filamentous fungi.

Catalysis

D-Glucose + Acceptor $\rightleftharpoons$ D-Glucono-δ-lactone + Reduced acceptor

Specification and Preparation

Activity:	FAD-GDH activity	≧ 360 u/mg (PMS-DCIP method)
Appearance:	Yellow powder, lyophilized	
Additive:	Not added	

Characteristics

1. Molecular weight: 180,000 (Gel filtration)
2. Isoelectric point: 6.5
3. Km: 15×10^{-3} M
4. Optimum pH: 7.0
5. pH stability: 4.5-6.0 (37°C, 30 min)
6. Optimum temperature: 55°C
7. Thermal stability: up to 40°C (pH 6.5, 20 min)
8. Activator: Triton X-100
9. Inhibitors: Ag^{+}, Hg^{2+}
10. Application: Used for the enzymatic determination of glucose in blood or urine by glucose sensor and others.

Expiration (Storage)

36 months from the date of analysis when stored at -20°C or below in a dry place under sealed conditions.

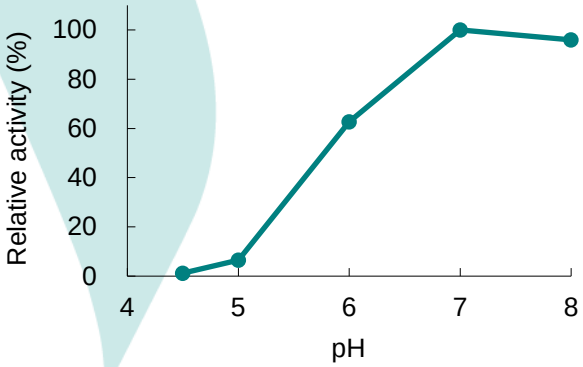
Safe Handling

1. Do not inhale.
2. In case of direct contact with skin or eyes, immediately wash or rinse with plenty of water.
3. Please refer to SDS for more details.

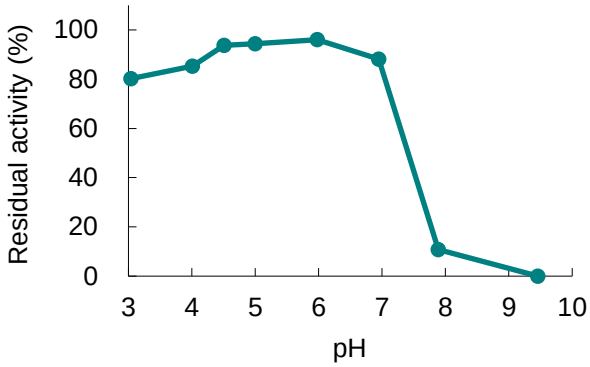
General properties

The following results demonstrate the activities of enzyme solution prepared in various buffers. Enzyme activity may vary under different experimental conditions.

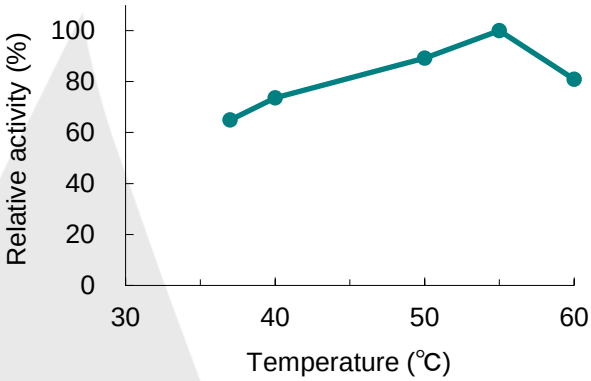
pH and Activity



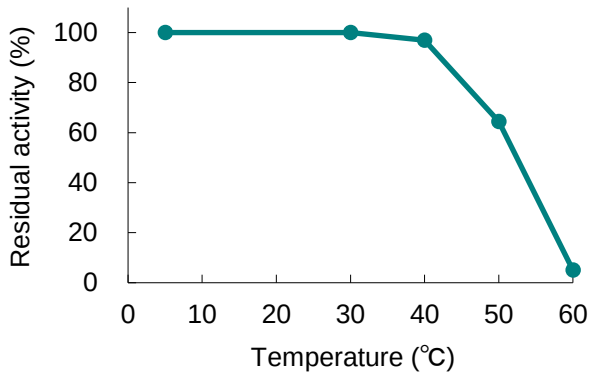
pH stability



Temperature and Activity



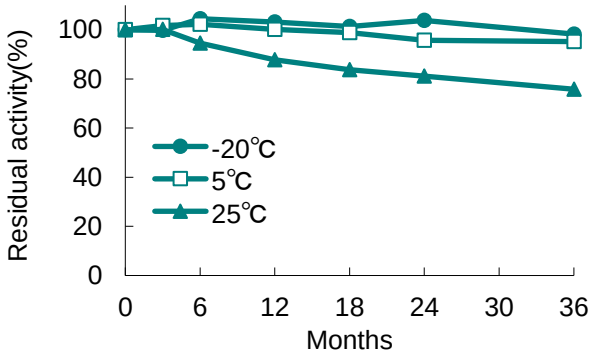
Thermostability



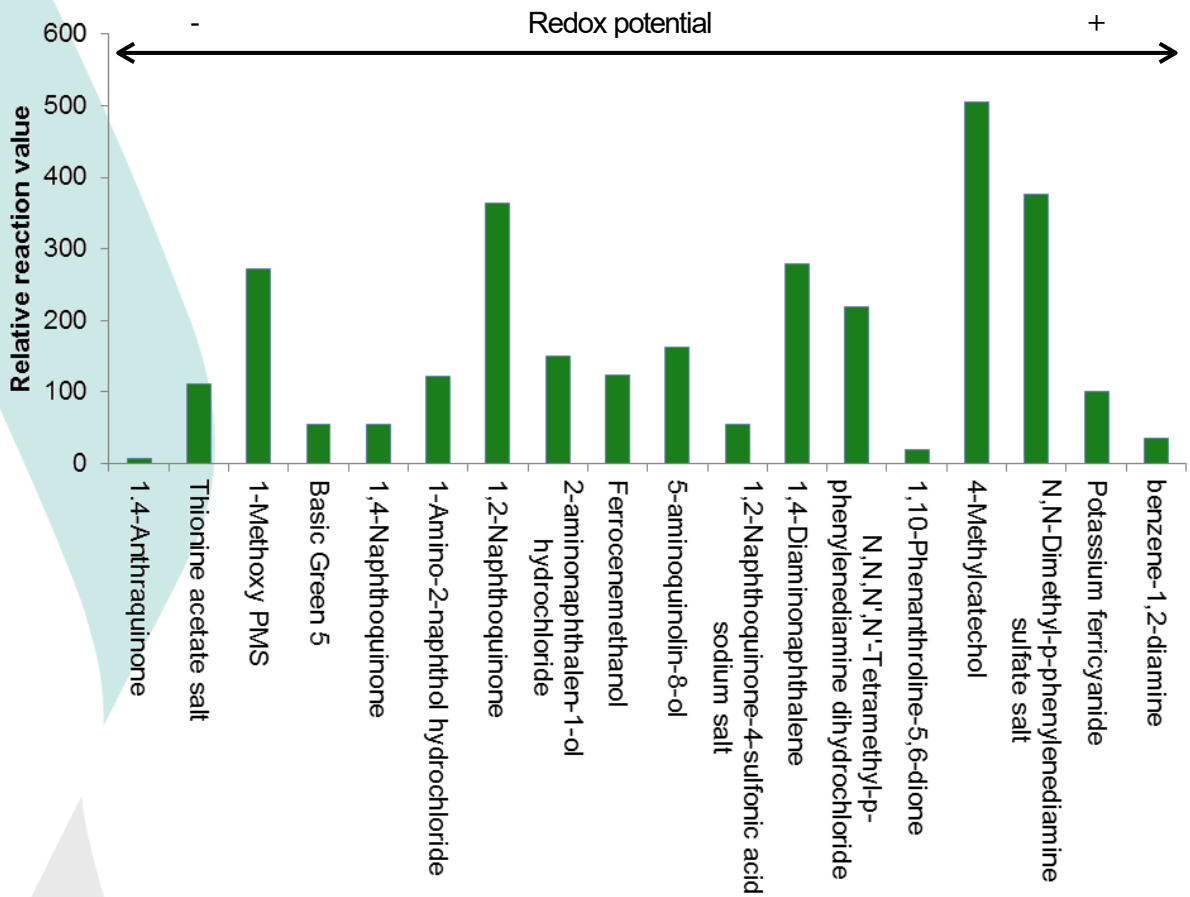
Substrate Specificity

Substrate	Relative Activity (%)
D-Glucose	100
D-Xylose	24
D-Maltose	0
D-Galactose	0

Stability (powder form)



Mediator Preferance



Assay method of FAD-GDH activity

Principle

FAD-dependent Glucose dehydrogenase

D-Glucose + PMS*1 → D-Glucono-δ-lactone + PMS*1 (reduced)

PMS*1 (reduced) + DCIP*2 → PMS + DCIP*2 (reduced)

*1: Phenazine methosulfate, *2: 2,6-Dichloroindophenol

The reduction of DCIP is measured at 600 nm by spectrophotometry.

Unit Definition

One unit is defined as the enzyme quantity which reduces one μ mole of DCIP per minute under the conditions described as follows.

Reagents

A. Triton X-100 solution (5%)
Weigh 5.0 g of Triton X-100 and dissolve in approx. 30 mL of deionized water while heating. Cool down until room temperature, fill up to 100 mL with deionized water. (Can be used for 1 month at room temperature)

B. Working solution (50 mM PIPES-NaOH buffer (pH 6.5) containing 0.1 % Triton X-100)
Weigh 1.51 g of PIPES and dissolve in approx. 70mL of deionized water. Add 2 mL of Triton X-100 solution (A), then adjust the pH to 6.5 with 4N NaOH. Fill up to 100 mL with deionized water. (Can be used for 1 month if kept refrigerated)

C. PMS solution (24 mM)
Weigh 73.5 mg of Phenazine methosulfate and dissolve in 10 mL of deionized water. (Can be used for 14 days in light-shielded and refrigerated storage)

D. DCIP solution (2 mM)
Weigh 6.5 mg of 2,6-Dichloroindophenol sodium salt n-hydrate and dissolve in 10 mL of deionized water. (Can be used for 14 days in light-shielded and refrigerated storage)

E. Substrate solution (1.0 M Glucose)
Weigh 3.6 g of D-glucose and dissolve in deionized water. Fill up to 20 ml with deionized water.
(Can be used for 14 days at room temperature)

F. Diluent
Weigh 6.8 g of KH_2PO_4 and dissolve in approx. 700 mL of deionized water. Add 20 mL of Triton X-100 solution (A), then adjust the pH to 5.5 with 4N NaOH. Fill up to 1000 mL with deionized water. (Can be used for 1 month if kept refrigerated)

G. Enzyme solution
Weigh out Glucose Dehydrogenase“Amano”8DC and dissolve in chilled Diluent (F).
Enzyme solution should be prepared so that the value of ΔOD/min becomes in the range from 0.035 to 0.100.

H. Mix solution
Mix 20.5 mL of Working solution (B), 2mL of PMS solution (C), 1 mL of DCIP solution (D) and 5.9 mL of Substrate solution (E). This solution should be kept in a light-proof tube to avoid exposure to light. (Can be used for 6 hours after preparation, in light shielded and refrigerated storage)

Procedure

Pipette 3 mL of Mix solution (H) into a disposable plastics cell (d=10 mm), and keep at 37±0.5℃ for 5 minutes. Then, pipette 0.1 mL of Enzyme solution (G) into the cell and mix well immediately. Keep the reaction mixture at 37±0.5℃. Exactly at 3 minutes and 5 minutes after the addition of Enzyme solution (G), measure the absorbances of the reaction mixture at 600 nm. (A3 and A5) As a blank, pipette Enzyme diluent (F) into another disposable plastics cell (d=10 mm) instead of Enzyme solution (G), and take the same procedure described above. (Ab3 and Ab5).

Calculation

$$\text{Glucose dehydrogenase activity (u/mg)} = \frac{(A3-A5)-(Ab3-Ab5)}{2} \times \frac{1}{16.8} \times 3.1 \times \frac{n}{0.1}$$

2: Reaction time
16.8: Millimolar extinction coefficient of DCIP at 600 nm
3.1: Volume of the reaction mixture
0.1: Volume of Enzyme solution
n: Dilution factor of Enzyme solution

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