Glucose Dehydrogenase “Amano” 2A

(β-D-Glucose: NAD(P)$^+$ 1-oxidoreductase, EC 1.1.1.47)

Glucose Dehydrogenase “Amano” 2A is a glucose dehydrogenase preparation manufactured by a unique fermentation process using a selected strain of bacteria.

Catalysis

β-D-Glucose + NAD(P)$^+$ ⇄ D-Glucono-δ-lactone + NAD(P)H + H$^+$

Specification and Preparation

Activity:	Glucose dehydrogenase activity	≧ 30 u/mg (Amano method)
Contaminants:	NADH oxidase activity	≦ 2×10^{-3}%
	Lactate dehydrogenase activity	≦ 2×10^{-3}%
Appearance:	White powder, lyophilized	
Additives:	BSA, NaCl	

Characteristics

1. Molecular weight: 105,000 (Gel filtration)
2. Isoelectric point: 4.5
3. Km: 0.8×10^{-2} M (Glucose)
   1.5×10^{-4} M (NAD)
   4.3×10^{-5} M (NADP)
4. Optimum pH: 8.0-9.0
5. pH stability: 5.0-8.0 (40°C, 90 min)
6. Optimum temperature: 50°C
7. Thermal stability: up to 80°C (pH 7.0, 50 min)
8. Inhibitors: Ag^+, Hg^{2+}
9. Stabilizers: Inorganic salts
10. Application: Used for the enzymatic determination of glucose in serum by coupling with mutarotase in clinical diagnosis.

Expiration (Storage)

24 months from the date of analysis when stored at 5°C or below in a dry place under sealed conditions.

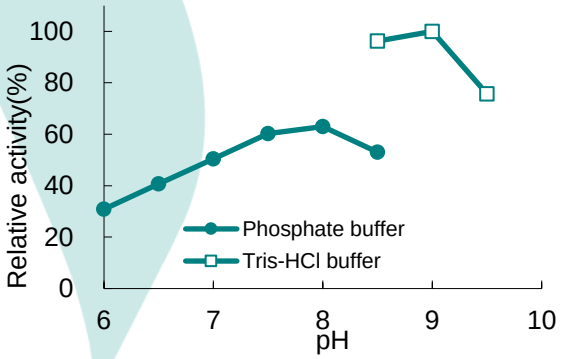
Safe Handling

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.

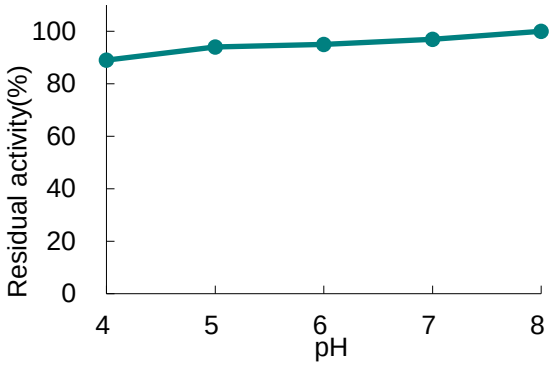
General properties

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

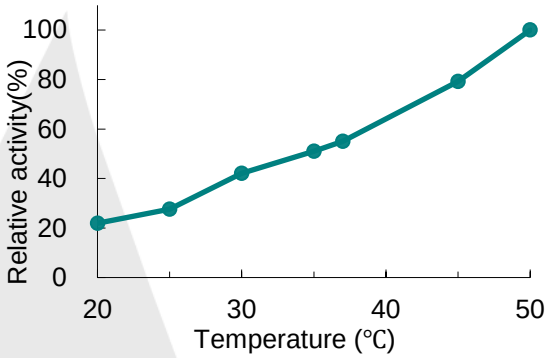
pH and Activity



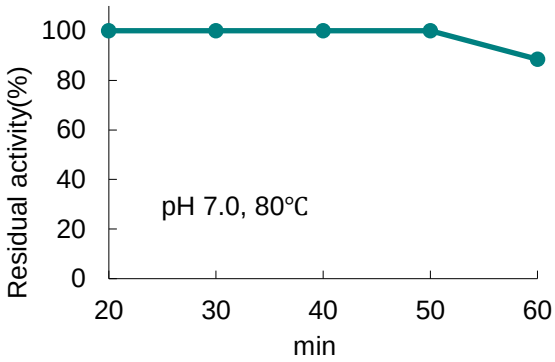
pH stability



Temperature and Activity



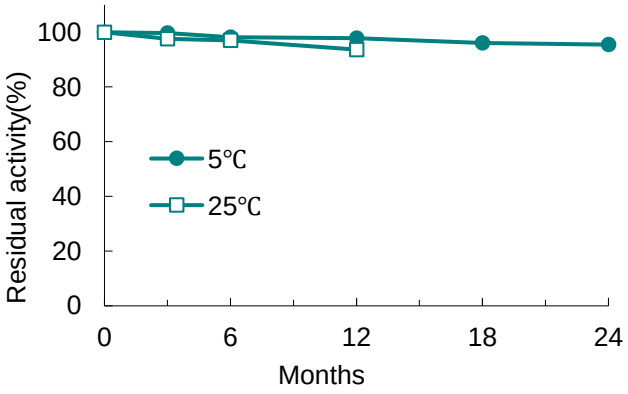
Thermostability



Substrate Specificity

Substrate	Relative Activity (%)
D-Glucose	100
D-Maltose	6
D-Xylose	20
D-Galactose	0
D-Mannose	10
D-Fructose	0
D-Sucrose	0

Stability (powder form)



Assay method of Glucose dehydrogenase activity

Principle

β-D-Glucose + NAD^+ $\xrightarrow{\text{Glucose dehydrogenase}}$ D-Glucono-δ-lactone + NADH + H^+

The appearance of NADH is measured at 340 nm by spectrophotometry.

Unit Definition

One unit is defined as the enzyme quantity which produces one μ mole of NADH per minute under the conditions described below.

Reagents

A. 0.1M Tris-HCl buffer (pH 8.0)
B. 0.1M Phosphate Buffer (KH_2PO_4-Na_2HPO_4, pH 7.0)
C. Substrate solution
   Weigh 6.75 g of glucose and dissolve in deionized water. Fill up to 25 ml with deionized water.
   Use Substrate solution at 30 minutes or later after preparation. (Can be used for 2 weeks at room temperature)
D. NAD solution
   Weigh 40 mg of β-NAD^+ (Oriental Yeast Co., Ltd.) and dissolve in 1 ml of deionized water.
   (Can be used for 1 week at 2-8℃)
E. Enzyme solution
   Weigh some of Glucose Dehydrogenase “Amano” 2A and dissolve in chilled 0.1 M Phosphate buffer (B).
   Enzyme solution should be prepared so that the value of ΔOD/minute becomes in the range of 0.100 ± 0.020.

Procedure

Pipette 2.7 ml of 0.1M Tris-HCl buffer (A), 0.2 ml of Substrate solution (C) and 0.1 ml of NAD solution (D) respectively into a quartz cell (d =10 mm), and keep at 25±0.5℃ for 5 minutes. Then, pipette 0.05 ml of Enzyme solution (E) into the quartz cell and mix well immediately. Keep the reaction mixture at 25±0.5℃. Exactly at 2 minutes and 5 minutes after the addition of Enzyme solution (E), measure the absorbances of the reaction mixture at 340 nm (A2 and A5). As a blank, pipette 0.1M Phosphate buffer (B) into another quartz cell (d =10 mm) instead of Enzyme solution (E), and take the same procedure described above (Ab2 and Ab5).

Calculation

$$\text{Glucose dehydrogenase activity (u/mg)} = \frac{(A5-A2)-(Ab5-Ab2)}{3} \times \frac{1}{6.22} \times 3.05 \times \frac{n}{0.05}$$

3: Reaction time
6.22: Millimolar absorption coefficient of NADH at 340 nm
3.05: Volume of the reaction mixture
0.05: Volume of Enzyme solution
n: Dilution factor of Enzyme solution

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