Glucose Oxidase “Amano” NA

(β-D-Glucose: oxygen 1-oxidoreductase, EC 1.1.3.4)

Glucose Oxidase “Amano” NA is a glucose oxidase preparation manufactured by a unique fermentation process using a selected strain of filamentous fungi.

Catalysis

β-D-Glucose + O_2 ⟶ D-Glucono-δ-lactone + H_2O_2

Specification and Preparation

Activity:	Glucose oxidase activity	≧ 180 u/mg (Amano method)
	The activity may increase up to 160%, if the raction mixture is saturated with oxygen.	
Contaminant:	Catalase activity	≦ 1 u/mg
Appearance:	Yellow powder, lyophilized	
Additive:	Not added	

Characteristics

1. Molecular weight: Ca. 180,000 (Gel filtration)
2. Isoelectric point: 4.3
3. Km: 1.9×10^{-2} M
4. Optimum pH: 6.0
5. pH stability: 5.0-7.0 (40°C, 6hrs)
6. Optimum temperature: 37°C
7. Thermal stability: up to 50°C (pH 7.0, 15 min)
8. Inhibitors: Ag^{+}, Hg^{2+}, Cu^{2+}
9. 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.

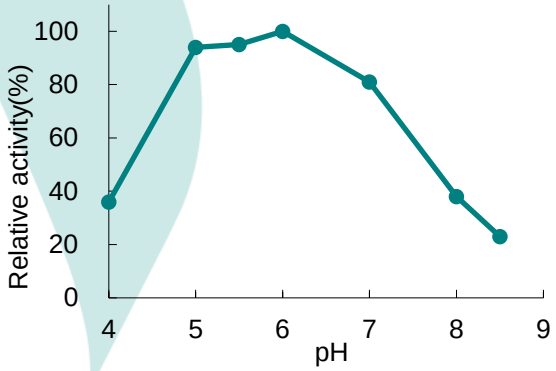
Safe Handling

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.

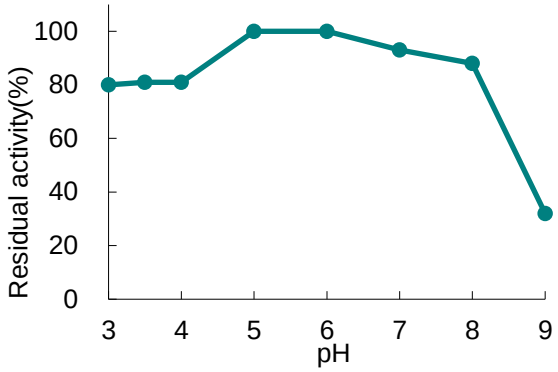
General properties

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

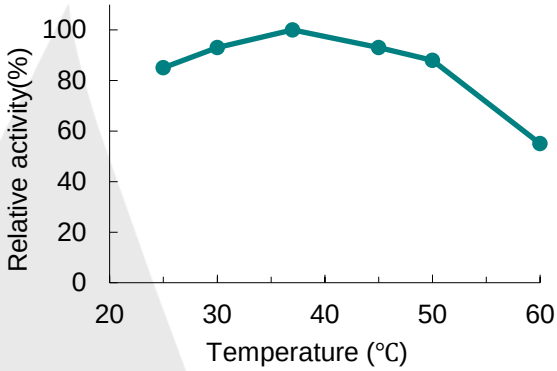
pH and Activity



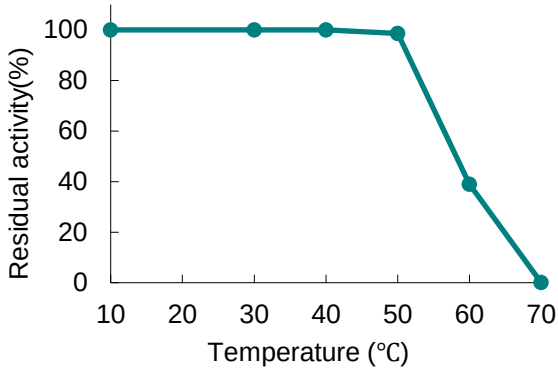
pH stability



Temperature and Activity



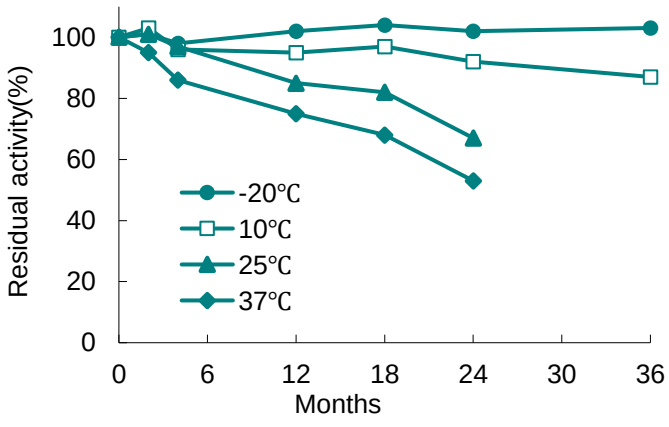
Thermostability



Substrate Specificity

Substrate	Relative Activity (%)
D-Glucose	100
2-Deoxy-D-glucose	11
4-o-Methyl-D-glucose	7
Mannose	3
Galactose	0
Xylose	0
Fructose	0

Stability (powder form)



Assay method of Glucose oxidase activity

Principle

$$\beta\text{-D-Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{D-Glucono-}\delta\text{-lactone} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4H_2O$$

The appearance of quinoneimine dye formed by coupling with 4-aminoantipyrine and phenol is measured at 500nm by spectrophotometry.

Unit Definition

One unit is defined as the enzyme quantity which oxidizes one μ mole of β-D-glucose per minute under the conditions described below.

Reagents

A. 4-Aminoantipyrine solution (4 mg/ml deionized water)
B. Triton X-100 solution (50 mg/ml deionized water)
C. Phenol solution (50mg/ml deionized water)
D. Peroxidase solution
   Dissolve 250 units of Peroxidase “Amano” 3 (Amano Enzyme) in 10 ml of deionized water.
   Keep in an ice bath. (Can be used for 11 days if kept refrigerated)
E. 0.1M Phosphate buffer (KH_2PO_4-NaOH, pH 7.0)
F. Phenol-buffer solution
   Weigh 1.36g of KH_2PO_4 and dissolve in 80 ml of deionized water. Add 3 ml of Phenol solution (C) and 3 ml of Triton X-100 solution (B), then adjust the pH to 7.0 with 4N NaOH. Fill up to 100 ml with deionized water. This reagent should be used after incubation at 25℃ for 24 hours. (Can be used for 2 weeks at 25℃)
G. Substrate solution (10% (w/v) D-glucose solution)
   (Can be used for 11 days at room temperature)
H. Enzyme solution
   Weigh some of Glucose Oxidase “Amano” NA and dissolve in chilled 0.1M Phosphate buffer (E).
   Enzyme solution should be prepared so that the value of ΔOD/minute becomes in the range of 0.030±0.005.

Procedure

Pipette 2.0 ml of Phenol-buffer solution (F), 0.5mL of Substrate solution (G), 0.5mL of Peroxidase solution (D) and 0.1 ml of 4-Aminoantipyrine solution (A) respectively into quartz cell (d=10 mm). Keep at 37±0.5℃ for 10 min. Then, pipette 0.1 ml of Enzyme solution (H) into the quartz cell and mix well immediately. Keep the reaction mixture at 37±0.5℃. Exactly at 2 minutes and 5 minutes after the addition of Enzyme solution, measure the absorbances of the reaction mixture at 500 nm (A2 and A5).
As a blank, pipette 0.1M Phosphate buffer (E) into another quartz cell instead of the Enzyme solution (H) and take the same procedure described above (Ab2 and Ab5).

Calculation

$$\text{Glucose Oxidase activity (u/mg)} = \frac{(A5-A2)-(Ab5-Ab2)}{3} \times \frac{3.2}{12.88} \times 2 \times \frac{n}{0.1} \times 1.339$$

3: Reaction time
12.88: Absorption coefficient of quinoneimine dye
2: Conversion factor (1 mole of quinoneimine dye equivalent to 2 mole of glucose)
3.2: Volume of the reaction mixture
0.1: Volume of Enzyme solution
n: Dilution factor of Enzyme solution
1.339: Coefficient (to original Amano method using O-dianisidine which is found to be a carcinogenic substance)

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