Glycerol Kinase“Amano”2

(ATP : Glycerol 3-phosphotransferase, EC 2.7.1.30)

Glycerol Kinase“Amano”2 is a glycerol kinase preparation manufactured by a unique fermentation process using a selected strain of bacteria.

Catalysis

Glycerol +ATP ⟶ Glycerol-3-phosphate + ADP

Specification and Preparation

Activity:	Glycerol kinase activity	≧ 10 u/mg (Amano method)
Contaminants:	ATPase activity	≦ 1.0×10^{-2}%
	Catalase activity	≦ 1.0×10^{-1}%
	Hexokinase activity	≦ 5.0×10^{-2}%
	NADH oxidase activity	≦ 1.0×10^{-2}%
Appearance:	White to light yellowish white powder, lyophilized	
Additive:	Sodium glutamate	

Characteristics

1. Molecular weight: 82,000 (Gel filtration)
2. Isoelectric point: 4.3
3. Km: 3.5×10^{-5} M (Glycerol)
   3.7×10^{-5} M (ATP)
4. Optimum pH: 10.0
5. pH stability: 5.0-9.5 (37°C, 1hr)
6. Optimum temperature: 50°C
7. Thermal stability: up to 50°C (pH 7.0, 15 min)
8. Inhibitors: SH reagents
9. Stabilizer: ATP
10. Application: Used for the enzymatic determination of triglyceride in serum by coupling with glycerophsphate dehydrogenase (G-3-PDH) and L-α-glycerophosphate oxidase (GPO) or pyruvate kinase and lactate dehydrogenase in clinical diagnosis.

Expiration (Storage)

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

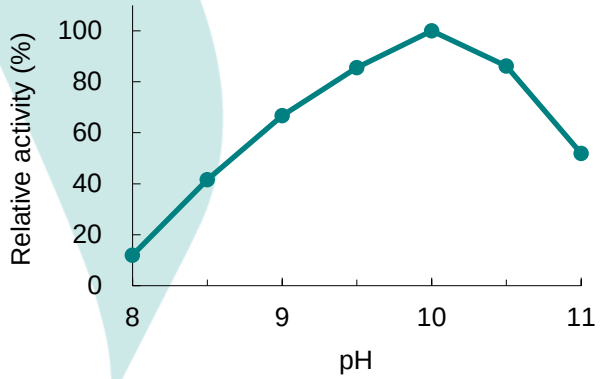
Safe Handling

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.

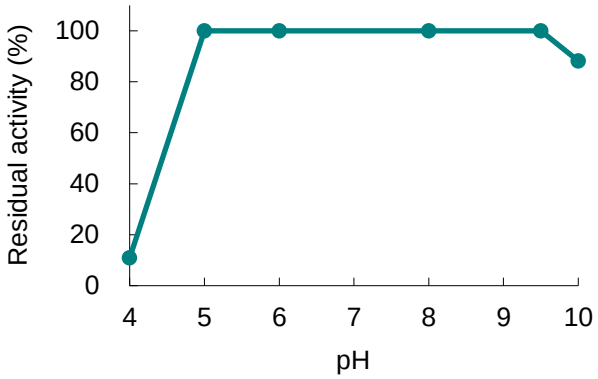
General properties

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

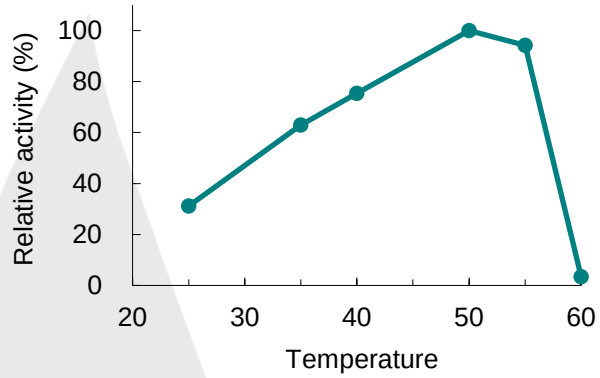
pH and Activity



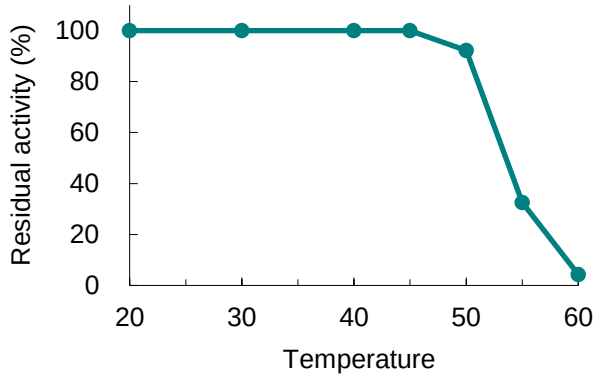
pH stability



Temperature and Activity



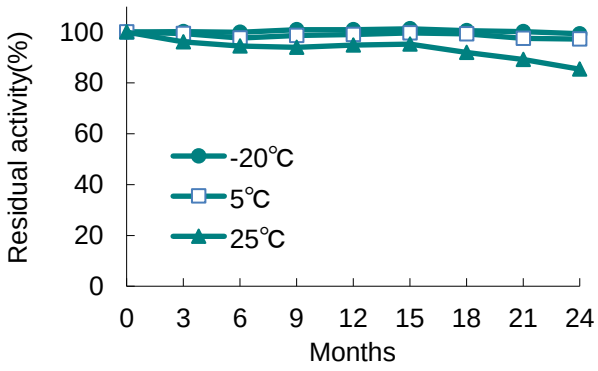
Thermostability



Substrate Specificity

Substrate	Relative Activity (%)
Glycerol	100
Dihydroxyacetone	97
DL-Glyceraldehyde	55

Stability (powder form)



Assay method of Glycerol kinase activity

Principle

$$\text{Glycerol + ATP} \xrightarrow[Mg^{2+}]{\text{Glycerol kinase}} \text{Glycerol-3-phosphate + ADP}$$

*G3PDH: Glycerol-3-phosphate dehydrogenase

$$\text{Glycerol-3-phosphate + NAD}^{+} \xrightarrow{\text{G3PDH*}} \text{Dihydroxyacetonephpsphate + NADH + H}^{+}$$

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. 0.2M Glycine-hydrazine buffer (containing 2 mM $MgCl_2$, pH 9.8)
Weigh 15 g of glycine, 51.1 g of hydrazine hydrate (98%, FUJIFILM Wako Pure Chemical Corporation) and 406 mg of $MgCl_2 \cdot 6H_2O$ then dissolve in 800 ml of deionized water. Adjust the pH to 9.8 at 25℃ with 1N KOH and fill up to 1000 ml with deionized water. (Can be used for 3 months if kept refrigerated)

B. 0.02M Phosphate buffer (KH_2PO_4-Na_2HPO_4, pH 7.0)

C. NAD/ATP solution
Weigh 10 mg of β-NAD (Oriental Yeast Co., Ltd.) and 30 mg of ATP・2Na (Oriental Yeast Co., Ltd.), then dissolve in 20 ml of 0.2M Glycine-hydrazine buffer (A). (Can be used for 5 days if kept refrigerated)

D. Substrate solution
Weigh 92 mg of glycerol (FUJIFILM Wako Pure Chemical Corporation) and dissolve in 0.2 M Glycine-hydrazine buffer (A). Fill up to 100 ml with 0.2 M Glycine-hydrazine buffer (A). (Can be used for 1 month if kept refrigerated)

E. G3PDH solution
(Produced by Roche Diagnostics GmbH)

F. Diluent
Weigh 303 mg of ATP・2Na (Oriental Yeast Co., Ltd.) and dissolve in 0.02M Phosphate buffer (B). Fill up to 500 ml with 0.02M Phosphate buffer (B). (Can be used for 10 days if kept refrigerated)

G. Enzyme solution
Weigh out Glycerol Kinase“Amano”2 and dissolve in chilled Diluent (F). 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 NAD/ATP solution (C), 1.0 ml of Substrate solution (D) and 0.02 ml of G3PDH solution (E) respectively into a quartz cell (d = 10mm) and keep at 25±0.5℃ for 5 minutes. Then, pipette 0.1 ml of Enzyme solution (G) 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 (G), measure the absorbances of the reaction mixture at 340 nm (A2 an A5). As a blank, pipette Diluent (F) into another quartz cell (d = 10 mm) instead of Enzyme solution (G) and take the same procedure described above (Ab2 and Ab5).

Calculation

$$\text{Glycerol kinase activity (u/mg)} = \frac{(A5-A2)-(Ab5-Ab2)}{3} \times \frac{1}{6.22} \times 3.12 \times \frac{n}{0.1}$$

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

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