Glutaminase “Amano”1

(Glutamine aminohydrolase, EC 3.5.1.2)

Glutaminase “Amano”1 is a glutaminase preparation manufactured by a unique fermentation process using a selected strain of bacteria.

Catalysis

L-Glutamine + H_2O ⟶ L-Glutamic acid + NH_3

Specification and Preparation

Activity:	Glutaminase activity	≧ 800 GTU/g (In-house method)
Appearance:	Light brown powder	
Additive:	Not added	

Characteristics

1. Molecular weight: 60,500 (Amino acid sequence)
2. Optimum pH: 6.2
3. pH stability: 5.0-9.0 (37°C, 16hrs)
4. Optimum temperature: 60-65°C
5. Thermal stability: up to 50°C (pH 6.0, 1hr)
6. Application: Used for the enzymatic determination of glutamine in serum by coupling with related enzymes in clinical diagnosis.

Expiration (Storage)

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

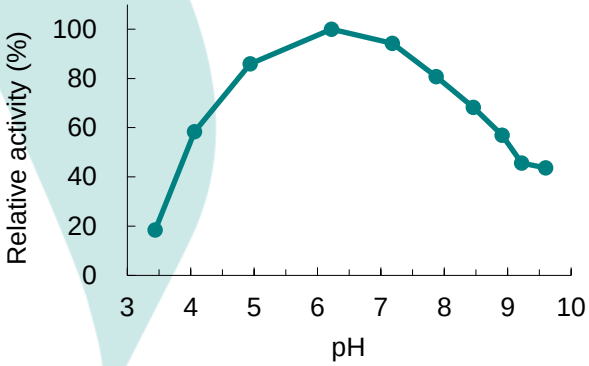
Safe Handling

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.

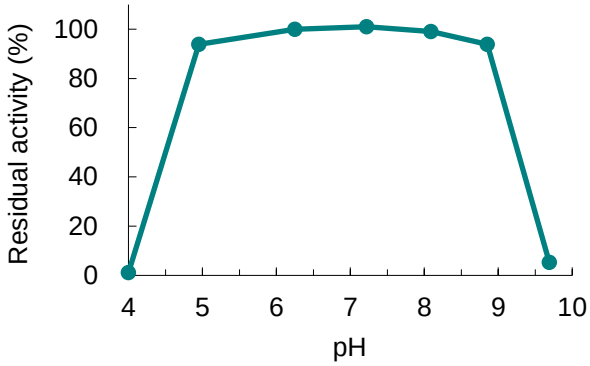
General properties

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

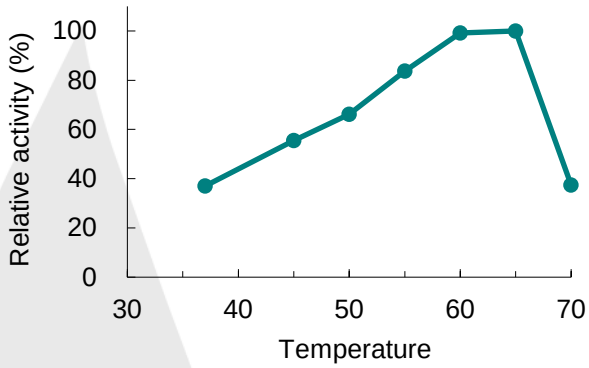
pH and Activity



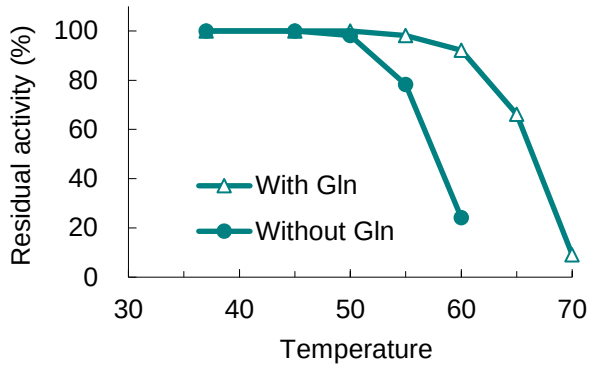
pH stability



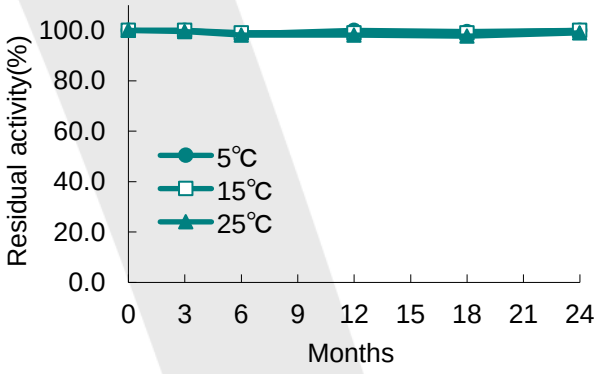
Temperature and Activity



Thermostability

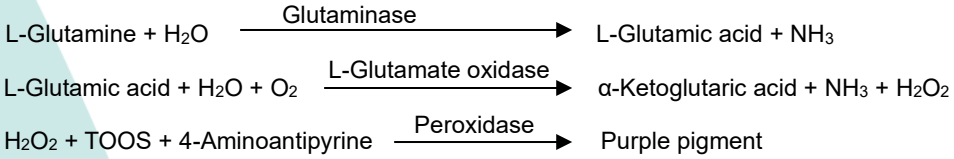


Stability (powder form)



Assay method of Glutaminase activity

Principle



The appearance of purple pigment is measured at 555 nm by spectrophotometry.

Unit Definition

One unit is defined as the enzyme quantity which generates one μ mole of L-glutamic acid per minute under the conditions described below.

Reagents

A. 1 M Acetic acid buffer (acetic acid – sodium acetate, pH6.0)

B. Triton X-100 solution (50mg/ml)

C. Sodium hydroxide solution

Weigh 3.0 g of sodium hydroxide in 80 ml of deionized water and cool. Fill up to 100 ml with deionized water.

D. Perchloric acid solution

Dilute 8.3 ml of perchloric acid (60%) to 100 ml with deionized water.

E. Diluent

Mix approx. 600 ml of deionized water, 1 ml of Triton X-100 solution (B), and 10 ml of 1M Acetic acid buffer (A), then fill up to 1000 ml with deionized water.

F. L-Glutamic acid Assay Kit YAMASA NEO (Yamasa Corporation)

F-1: R1 enzyme reagent

F-2: R2 enzyme reagent

F-3: Glutamic acid standard solution (250 mg/L)

G. Substrate solution

Weigh 1.00 g of L-glutamine and dissolved in approx. 35ml of deionized water, then add 5 ml of 1 M Acetic acid buffer (A). Fill up to 50 ml with deionized water.

H. Enzyme solution

Weigh out Glutaminase “Amano”1 and dissolve in Diluent (E). Enzyme solution should be prepared so that the value of (A_S-A_{SB}) becomes in the range of 0.2-0.5 (approx. 0.45-1.10 GTU/ml).

Procedure

<Glutaminase reaction>
Pipette 1.0 ml of Enzyme solution (H) into a test tube and keep at 37 ± 0.5℃ for 5 minutes. Then, add 1 ml of preheated (37 ± 0.5℃) Substrate solution (G) into the test tube and mix, keep the mixture at 37 ± 0.5℃. Exactly after 10 minutes, add Perchloric acid solution (D) and mix, then place in iced water. After 1 minute or more, add 1 ml of Sodium hydroxide solution (C) and mix (Reaction solution).

<L-Glutamic acid Assay Kit>
Pipette 1800 µl of R1 enzyme reagent (F-1) and 40 µl of Reaction solution and mix in another test tube, then pipette 1800 µl of R2 enzyme reagent (F-2) and mix. Exactly after 20 minutes at room temperature, measure the absorbances of the mixture at 555 nm (A_S, Reaction mixture).

<Blank measurement>
As the enzyme blank, pipette 1 ml of Enzyme solution (H) in an empty test tube, add 1 ml of Perchloric acid solution (D) and mix, and keep 37 ± 0.5℃ for 5min. Add 1ml of Substrate solution (G) and immediately place in iced water. After 1 minute or more, add 1ml of Sodium hydroxide solution (C) and mix. After then, follow the procedure of L-Glutamic acid Assay Kit and measure the absorbance at 555nm (A_{SB}).
Also using Glutamic acid standard solution (F-3) or water, measure the absorbance following the same procedure as for the enzyme blank (A_R, A_{RB}).

Calculation

$$\text{Glutaminase activity (GTU/g)} = \frac{(A_S - A_{SB})}{(A_R - A_{RB})} \times 250 \times 4 \times \frac{1}{147} \times \frac{1}{10} \times n$$

A_S: Absorbance of reaction mixture
A_{SB}: Absorbance of enzyme blank reaction mixture
A_R: Absorbance of glutamic acid standard reaction mixture
A_{RB}: Absorbance of water blank reaction mixture
250: Concentration of L-Glutamic acid standard solution
4: Volume of reaction mixture
147: Molecular weight of L-Glutamic acid
10: Reaction time
N: Dilution factor of Enzyme solution

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