Glutamine Synthetase (GST)

from microorganism

L-Glutamate : ammonia ligase (ADP-forming), EC 6.3.1.2

$$ATP + \text{L-Glutamate} + NH_3 \longrightarrow ADP + \text{Orthophosphate} + \text{L-Glutamine}$$

SPECIFICATION

Appearance	light yellow lyophilizate	
Activity	≧7 U/mg lyophilizate	
Contaminants	catalase	≦0.5%
Stabilizer	sucrose	
Storage	at −20°C	

PROPERTIES

Molecular weight	*ca*. 900 kDa (gel filtration)	
Structure	57 kDa (SDS-PAGE)	
Isoelectric point	6.5	
Michaelis constants	1.5×10^{-2} M (L-glutamate)	
	1.3×10^{-4} M (ammonia)	
	8.7×10^{-4} M (ATP)	
pH Optimum	7.0	(Fig. 1)
pH Stability	6.5–9.5	(Fig. 2)
Optimum temperature	60°C	(Fig. 3)
Thermal stability	below 40°C	(Fig. 4)
Inhibitors	methionine sulfoximine, carbamyl phosphate	
Activators	Mg^{2+}, Mn^{2+}	
Specificity	L-glutamate (100), D-glutamate (0.8)	
	NH_3 (100), NH_2OH (12)	
	ATP (100), GTP (2.5)	

ASSAY PROCEDURE

Principle

$$\text{ATP} + \text{L-Glutamate} + NH_3 \xrightarrow{\text{glutamine synthetase}} \text{ADP} + \text{Phosphate} + \text{L-Glutamine}$$

The appearance of phosphate is measured spectrophotometrically at 660 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 μmol of phosphate per min at 37°C and pH 7.0 under the conditions described below.

Reagents

A. Imidazole–HCl buffer, 1.0 M; pH 7.0: dissolve 6.81 g of imidazole in 80 ml of distilled water, adjust to pH 7.0 with 4 N HCl and dilute with distilled water to 100 ml.
B. ATP solution, 0.12 M: dissolve 0.726 g of ATP·Na_2·$3H_2O$ in 8 ml of distilled water, adjust to pH 7.0 with 1 N NaOH and dilute with distilled water to 10 ml.
C. L-Glutamate solution, 2.0 M: dissolve 37.4 g of sodium L-glutamate in 80 ml of distilled water, adjust to pH 7.0 with 1 N NaOH and dilute with distilled water to 100 ml.
D. NH_4Cl solution, 1.0 M: 5.35 g of NH_4Cl/100 ml of distilled water.
E. $MgCl_2$ solution, 1.67 M: 34.0 g of $MgCl_2$·$6H_2O$/100 ml of distilled water.
F. $FeSO_4$ solution, 29 mM: 0.8 g of $FeSO_4$·$7H_2O$/100 ml of 0.3 N H_2SO_4 (prepare freshly).
G. Ammonium molybdate reagent, 53 mM: 6.6 g of $(NH_4)_6Mo_7O_{24}$·$4H_2O$/100 ml of 7.5 N H_2SO_4.

Sample: dissolve the lyophilized enzyme to a volume activity of 0.08–0.18 U/ml in ice-cold 50 mM imidazole–HCl buffer, pH 7.0, immediately before measurement.

Procedure

1. Prepare the following reaction mixture.

1.0 ml	Imidazole–HCl buffer	(Reagent A)
2.5 ml	ATP solution	(Reagent B)
2.0 ml	L-Glutamate solution	(Reagent C)
1.0 ml	NH_4Cl solution	(Reagent D)
0.6 ml	$MgCl_2$ solution	(Reagent E)
2.9 ml	Distilled water	

2. Pipette 0.2 ml of the reaction mixture and 0.1 ml of distilled water in a test tube.
3. Equilibrate at 37°C for about 5 min.
4. Add 0.1 ml of sample and incubate for 10 min at 37°C.
5. Add 1.8 ml of $FeSO_4$ solution (Reagent F) to stop the reaction, and then add 0.15 ml of ammonium molybdate reagent (Reagent G).
6. Read the absorbance at 660 nm in a cuvette (light path: 1 cm) (A_S).
   The blank solution is prepared by reversing the sequence of the addition of sample and $FeSO_4$ solution (Reagent F) (A_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(A_S - A_0) \times 0.4\,(\text{ml}) \times df}{0.556 \times 0.1(\text{ml}) \times 10(\text{min})} = \Delta A \times 0.719 \times df$$

Weight activity (U/mg) = (U/ml) × 1/*C*

0.556 : Millimolar extinction coefficient of phosphate under the assay conditions ($cm^2/\mu mol$)
df : Dilution factor
C : Content of glutamine synthetase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of ammonia and ATP in clinical analysis.

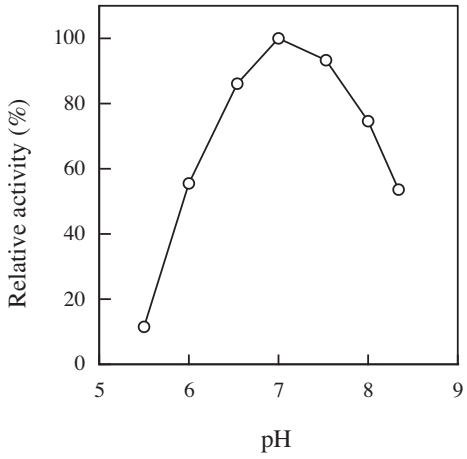
$$\text{ATP} + \text{L-Glutamate} + NH_3 \xrightarrow{\text{glutamine synthetase}} \text{ADP} + \text{Phosphate} + \text{L-Glutamine}$$

REFERENCE

Ebner, E. *et al*., "Methods in Enzymology," Vol. 17A, Academic Press, New York, 1970, pp. 910–922.

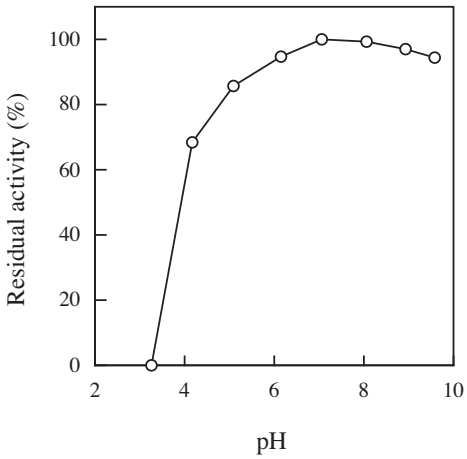
EXPERIMENTAL DATA

Fig. 1 pH Optimum



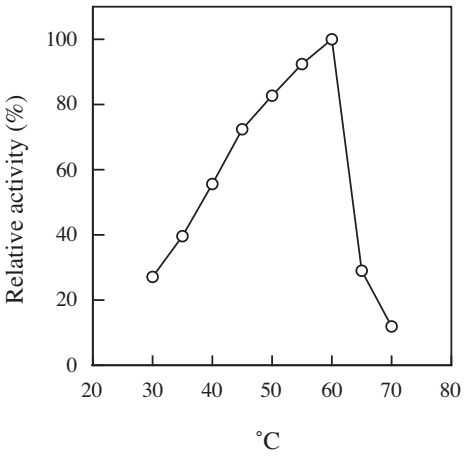
Buffer: 50 mM Tris–imidazole–acetate buffer

Fig. 2 pH Stability



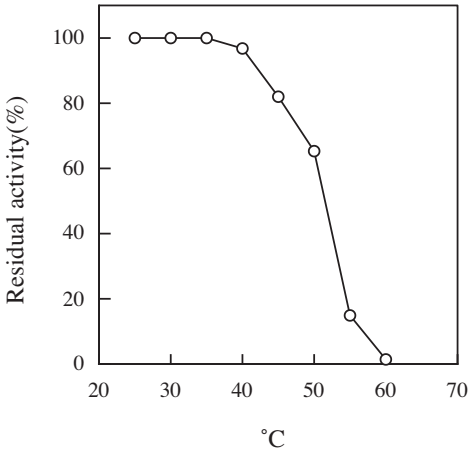
Treatment: 50 mM Tris–imidazole–acetate buffer, 5°C, 3 days

Fig. 3 Optimum temperature



Buffer: 50 mM imidazole–HCl buffer, pH 7.0

Fig. 4 Thermal stability



Treatment: 50 mM imidazole–HCl buffer, pH 7.0, 10 min