Glutamine Synthetase (GST)

from microorganism

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

\[
L-\text{Glutamate} + \text{NH}_3 \rightleftharpoons \text{ADP} + \text{Orthophosphate} + L-\text{Glutamine}
\]

SPECIFICATION
- Appearance: light yellow lyophilizate
- Activity: \( \geq 7 \text{ U/mg lyophilizate} \)
- Contaminants: catalase \( \leq 0.5\% \)
- Stabilizer: sucrose
- Storage: at \(-20^\circ\text{C}\)

PROPERTIES
- Molecular weight: ca. 900 kDa (gel filtration)
- Structure: 57 kDa (SDS-PAGE)
- Isoelectric point: 6.5
- Michaelis constants:
  - \( 1.5 \times 10^{-2} \text{ M} \) (L-glutamate)
  - \( 1.3 \times 10^{-4} \text{ M} \) (ammonia)
  - \( 8.7 \times 10^{-4} \text{ M} \) (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: \( \text{Mg}^{2+}, \text{Mn}^{2+} \)
- Specificity:
  - L-glutamate (100), D-glutamate (0.8)
  - \( \text{NH}_3 \) (100), \( \text{NH}_2\text{OH} \) (12)
  - ATP (100), GTP (2.5)
ASSAY PROCEDURE

Principle

\[
\text{ATP} + \text{l-Glutamate} + \text{NH}_3 \xrightarrow{\text{glutamine synthetase}} \text{ADP} + \text{Phosphate} + \text{l-Glutamine}
\]

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·Na₂·3H₂O 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. NH₄Cl solution, 1.0 M: 5.35 g of NH₄Cl/100 ml of distilled water.

E. MgCl₂ solution, 1.67 M: 34.0 g of MgCl₂·6H₂O/100 ml of distilled water.

F. FeSO₄ solution, 29 mM: 0.8 g of FeSO₄·7H₂O/100 ml of 0.3 N H₂SO₄ (prepare freshly).

G. Ammonium molybdate reagent, 53 mM: 6.6 g of (NH₄)₆Mo₇O₂₄·4H₂O/100 ml of 7.5 N H₂SO₄.

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 NH₄Cl solution (Reagent D)
   - 0.6 ml MgCl₂ 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 FeSO₄ 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) (Aₚ). The blank solution is prepared by reversing the sequence of the addition of sample and FeSO₄ solution (Reagent F) (A₀).
**Calculation**

Activity can be calculated by using the following formula:

\[
\text{Volume activity (U/ml)} = \frac{(A_k - A_b) \times 0.4 (\text{ml}) \times df}{0.556 \times 0.1(\text{ml}) \times 10(\text{min})} = \Delta t \times 0.719 \times df
\]

\[
\text{Weight activity (U/mg)} = (\text{U/ml}) \times \frac{1}{C}
\]

0.556: Millimolar extinction coefficient of phosphate under the assay conditions (cm²/µmol)

\( df \) : 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.

\[
\text{ATP + L-Glutamate + NH}_3 \xrightarrow{\text{glutamine synthetase}} \text{ADP + Phosphate + L-Glutamine}
\]

**REFERENCE**

EXPERIMENTAL DATA

Fig. 1  pH Optimum

![Graph showing pH optimum with relative activity (%) on the y-axis and pH on the x-axis.]

Buffer: 50 mM Tris–imidazole–acetate buffer

Fig. 2  pH Stability

![Graph showing pH stability with residual activity (%) on the y-axis and pH on the x-axis.]

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

Fig. 3  Optimum temperature

![Graph showing optimum temperature with relative activity (%) on the y-axis and temperature on the x-axis.]

Buffer: 50 mM imidazole–HCl buffer, pH 7.0

Fig. 4  Thermal stability

![Graph showing thermal stability with residual activity (%) on the y-axis and temperature on the x-axis.]

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