Glutamate Synthase Assay Kit

Catalog No.: abx096004

Size: 96 tests

Range: 8.84 U/L – 321.05 U/L

Sensitivity: 8.84 U/L

Storage: Store at -20°C in the dark.

Application: The qualitative detection of Glutamate Synthase activity in serum, plasma and tissue homogenates.

Principle of the Assay: Glutamate synthase catalyzes the production of glutamic acid by the oxidation of NADH. The rate of consumption of NADH, which has an absorbance peak at 340 nm, is proportional to the activity of Glutamate synthase. The Optical Density (OD) is measured spectrophotometrically at 340 nm in a microplate reader, from which the activity of Glutamate synthase can be determined.

Kit components

1. 96 well microplate
2. Extraction Buffer: 2 × 50 ml
3. Reaction Buffer: 26 ml
4. Substrate A: 2 vials
5. Substrate B: 2 vials
6. Chromogenic Reagent: 2 vials
7. Plate Sealer: 2

Materials required but not provided

1. Pipettes and pipette tips
2. Centrifuge and centrifuge tubes
3. Double-distilled water
4. PBS (0.01 M, pH 7.4)
5. Microplate reader (340 nm)
6. Vortex mixer
7. Mechanical homogenizer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and Plasma:** Serum and plasma samples can be tested directly. If not tested immediately, samples can be stored at -80°C for up to 1 month.
- **Tissue homogenates:** Weigh at least 20 mg of tissue and wash in cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue, add 180 µl of Extraction Buffer. Homogenize manually, using a mechanical homogenizer at 4°C. Centrifuge the homogenate at 10,000 x g for 10 minutes at 4°C. Collect the supernatant, keep on ice and assay immediately.

Note: To calculate Glutamate Synthase activity in tissue homogenates using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (**abx097193**).

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Buffer, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Rat kidney tissue homogenate	2 – 4
10% Rat liver tissue homogenate	2 – 4
10% Rat heart tissue homogenate	2 – 4
10% Mouse liver tissue homogenate	2 – 4
Cow serum	1
10% *Pleurotus cornucopiae* tissue homogenate	1
10% Beech Mushroom tissue homogenate	1

Notes:

- Store frozen samples undiluted. Thaw samples once ready to analyze. Avoid repeated freeze/thaw cycles.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- If a sample is not indicated in the manual's applications, a preliminary experiment to determine the suitability of the kit will be required.

2. Reagent Preparation

- **Reaction Working Solution:** Add 12.5 ml of Reaction Buffer to 1 vial of Substrate A and mix thoroughly to dissolve. Add the resultant solution to 1 vial of Substrate B and mix thoroughly to dissolve. Add the resultant solution to 1 vial of Chromogenic Reagent and mix thoroughly to dissolve. This solution should be prepared immediately before carrying out the assay and kept in the dark.

Notes:

- Equilibrate all reagents to room temperature before use.

B. Assay Protocol

1. Mark positions on the 96-well microplate for each standard, blank, sample. *It is recommended to prepare all wells in duplicate.*
2. Aliquot 20 µl of each sample into the corresponding sample wells.
3. Aliquot 200 µl of Reaction Working Solution to the sample wells.
4. Mix thoroughly for 5 seconds then immediately measure the OD of each well at 340 nm (A_1).
5. Incubate for exactly 4 minutes at room temperature.
6. Measure the OD of each well at 340 nm (A_2).

Notes:

- For accurate time measurements, it is recommended to measure no more than 4 samples at one time.

C. Calculations

Serum and Plasma:

1 unit (U) is defined as the quantity of enzyme in a 1 L reaction system that will decompose 1 µmol of NADH per minute at 25°C.

$$\textbf{Glutamate synthase activity (U/L)} = \frac{(\mathbf{A_1 - A_2})}{\boldsymbol{\varepsilon \times d}} \times \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample} \times T}} \times \mathbf{F} \times \mathbf{10^6}$$

Tissue homogenates:

1 unit (U) is defined as the quantity of enzyme in a 1 g reaction system that will decompose 1 µmol of NADH per minute at 25°C.

$$\textbf{Glutamate synthase activity (U/g protein)} = \frac{(\mathbf{A_1 - A_2})}{\boldsymbol{\varepsilon \times d}} \times \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample} \times T \times C_{Protein}}} \times \mathbf{F} \times \mathbf{10^6}$$

where:

$\mathbf{A_1}$	absorbance of the sample measured at 0 minutes
$\mathbf{A_2}$	absorbance of the sample measured at 4 minutes
$\boldsymbol{\varepsilon}$	Molar extinction coefficient of NADH (6.22×10^3 L/mol/cm)
$\mathbf{d}$	Optical path (0.65 cm)
$\mathbf{V_{Sample}}$	Total volume of sample used (0.02 ml)
$\mathbf{V_{Total}}$	Total volume of the reaction (0.22 ml)
$\mathbf{C_{Protein}}$	Concentration of protein in the sample (g/L)
$\mathbf{T}$	Reaction time (4 minutes)
$\mathbf{F}$	Sample dilution factor

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.