Glutamate Synthase Assay Kit

Catalog No: abx096004

Size: 96T

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 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

- 96 well microplate
- Extraction Buffer: 2 × 50 ml
- Reaction Buffer: 26 ml
- Substrate A: 2 vials
- Substrate B: 2 vials
- Detection Reagent: 2 vials
- Plate Sealer: 2

Materials Required But Not Provided

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- 1.5 ml tubes
- Double Distilled water
- Absorbent filter papers
- Microplate reader (340 nm)
- Vortex

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Protocol

A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

- **Serum and plasma samples:** Prepare using conventional techniques, and may be detected directly. If samples show turbidimetry, centrifuge at 12,000 × g for 10 minutes.
- **Tissue homogenates:** Weigh the tissue homogenate. For each 0.1 g of homogenate, add 0.9 ml Extraction Buffer. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 × g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration of the supernatant should be determined separately.

Samples should be diluted within the detection range of the kit using Extraction Buffer. The recommended dilution factors for various samples are given below 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 analyse. 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.

B. Reagent Preparation

Reaction Working Solution: Dissolve a vial of Substrate A with 12.5 ml of Reaction Buffer. Mix fully, then use the resultant solution to dissolve a vial of Substrate B. Mix fully, then use the resultant solution to dissolve a vial of Detection Reagent and mix fully. Prepare immediately before use and keep in the dark.

C. Assay Protocol

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate.

1. Set sample wells and label accordingly.
2. Aliquot 20 µl of sample and 200 µl of Reaction Working Solution to the sample wells.
3. Mix orbital shaker, then measure the absorbance of each well at 340 nm (A_1).
4. Incubate for exactly 4 min at 25°C.
5. Measure the absorbance 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.

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D. Calculations

Serum/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.

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

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.

$$\textbf{Glutamate synthase activity}\ (\mathbf{U/g}) = \frac{(\mathbf{A_1} - \mathbf{A_2})}{\boldsymbol{\varepsilon} \times \boldsymbol{d}} \times \frac{\mathbf{V_{total}}}{\mathbf{V_{sample}} \times \boldsymbol{T} \times \mathbf{C_{pr}}} \times \boldsymbol{f} \times \mathbf{10^6}$$

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)
$\boldsymbol{d}$	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)
$\boldsymbol{C_{pr}}$	concentration of protein in the sample (g/L)
$\boldsymbol{T}$	reaction time (4 minutes)
$\boldsymbol{f}$	sample dilution factor