Product Manual

Glutamate Assay Kit (Colorimetric)

Catalog Number

MET-5080 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Glutamate is a non-essential amino acid that has a key metabolic role in processes such as the citric acid cycle and removal of excess nitrogen waste. In its monosodium form (MSG), glutamate is well known as a flavor enhancer. Glutamate has also been identified as one of the major excitatory neurotransmitters of the mammalian brain. Glutamate is involved in learning and memory, and long-term potentiation occurs at glutaminergic synapses. In addition, glutamate helps to regulate growth cones and synaptogenesis. Postsynaptically, glutamate has been suggested to activate the NMDA, AMPA, and kainite receptors. Damage and/or death to nerve cells due to excessive glutamate release and deficits in uptake have been correlated with diseases such as amyotrophic lateral sclerosis, lathyrism, and Alzheimer's disease as well as stroke, autism, and some forms of intellectual disability.

Assay Principle

The Glutamate Assay Kit is a sensitive, quantitative colorimetric assay for glutamate. The provided reagents are sufficient for the evaluation of 200 assays*. The unknown samples or Glutamate standards are added to a 96 well plate followed by the Colorimetric Probe Mix containing WST-1, an electron mediator, and L-Glutamic Dehydrogenase (GDH). During a brief incubation the WST-1 is converted to the formazan form (Figure 1) and the absorbance of the plate is read at 450 nm. The content of Glutamate in the unknown samples is determined by comparison with a predetermined Glutamate standard curve.

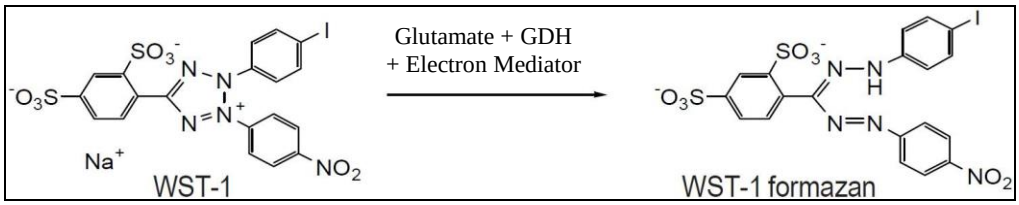

Figure 1. Assay Principle.

Note: Each sample replicate requires 2 assays, one treated with L-glutamic dehydrogenase (+GDH) and one without (-GDH). Glutamate levels are calculated from the difference in OD readings from the 2 wells.

Related Products

1. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
2. MET-5056: Branched Chain Amino Acid Assay Kit (Colorimetric)
3. MET-5125: Pyruvate Assay Kit (Colorimetric)
4. MET-5070: Glycine Assay Kit (Fluorometric)
5. MET-5130: Lysine Assay Kit (Colorimetric)

Kit Components

1. 10X Colorimetric Probe (Part No. 50801C): Two 2 mL amber vials.
2. L-Glutamate Standard (Part No. 50805C): One 100 µL vial at 200 mM.
3. 10X Assay Buffer (Part No. 50802A): One 30 mL bottle.
4. 50X NAD+ (Part No. 50803D): One 800 µL vial.
5. L-Glutamic Dehydrogenase (100X) (Part No. 50804C): One 400 µL vial at 125 U/mL

Note: One unit is defined as the amount of enzyme that reduces 1 µmol of α-ketoglutarate to L-glutamate per minute at 25 ºC and pH 7.3.

Materials Not Supplied

1. Distilled or deionized water
2. Standard 96-well clear microtiter plate

Storage

Upon receipt, store the 10X Assay Buffer at room temperature. Store the 50X NAD+ at -80°C. Store all remaining components at -20ºC. The 10X Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at room temperature.
- Reaction Mix: Dilute the 10X Colorimetric Probe, the L-Glutamic Dehydrogenase (100X) and the 50X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 20 assays add 400 µL of 10X Colorimetric Probe, 40 µL of L-Glutamic Dehydrogenase (100X), and 80 µL of 50X NAD+ to 3.48 mL of 1X Assay Buffer.

Note: Scale down the described example appropriately and prepare only enough for immediate use.

- Control Mix: Dilute both the 10X Colorimetric Probe and the 50X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 20 assays add 400 µL of 10X Colorimetric Probe, and 80 µL of 50X NAD+ to 3.52 mL of 1X Assay Buffer.

Note: Scale down the described example appropriately and prepare only enough for immediate use.

Preparation of Samples

Notes: All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with unknown samples.

- Cell culture supernatants: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary. Prepare the Glutamate standard curve in the same non-conditioned media.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates can be assayed undiluted or diluted as necessary in deionized water.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary in deionized water.

Preparation of Standard Curve

Prepare fresh Glutamate standards before use.

1. First, **dilute the stock L-Glutamate Standard 200 mM solution 1:10 in 1X Assay Buffer for a 20 mM Glutamate Solution**. (e.g., add 5 µL of the stock 200 mM L-Glutamate Standard to 45 µL of 1X Assay Buffer). **Vortex thoroughly**.

2. Use the 20 mM Glutamate Solution to prepare a series of the remaining Glutamate standards according to Table 1.

Standard Tubes	20 mM Glutamate Solution (µL)	1X Assay Buffer (µL)	Glutamate (µM)
1	10	490	400
2	250 of Tube #1	250	200
3	250 of Tube #2	250	100
4	250 of Tube #3	250	50
5	250 of Tube #4	250	25
6	250 of Tube #5	250	12.5
7	250 of Tube #6	250	6.25
8	0	250	0

Table 1. Preparation of Glutamate Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

Note: Each sample replicate requires two paired wells, one to be treated with GDH (Reaction Mix) and one without the enzyme (Control Mix) to measure endogenous sample background.

2. Add 50 µL of each sample (Glutamate standard or unknown) into wells of a 96 well plate.
3. Add 200 µL of Reaction Mix to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.
4. Add 200 µL of Control Mix to the other half of the paired sample wells and mix thoroughly.
5. Incubate at room temperature for 60 minutes on an orbital shaker.
6. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Glutamate Assay (Colorimetric) results. One should use the data below for reference only. This data should not be used to interpret actual results.

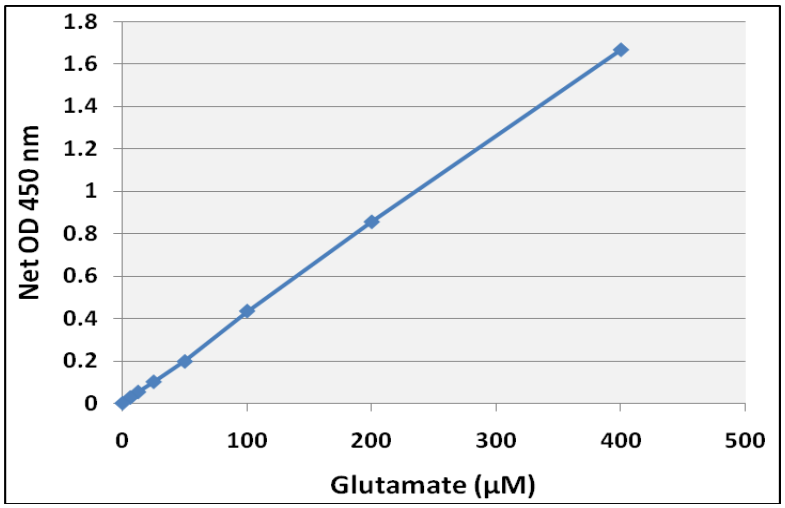

Figure 2. Glutamate Standard Curve.

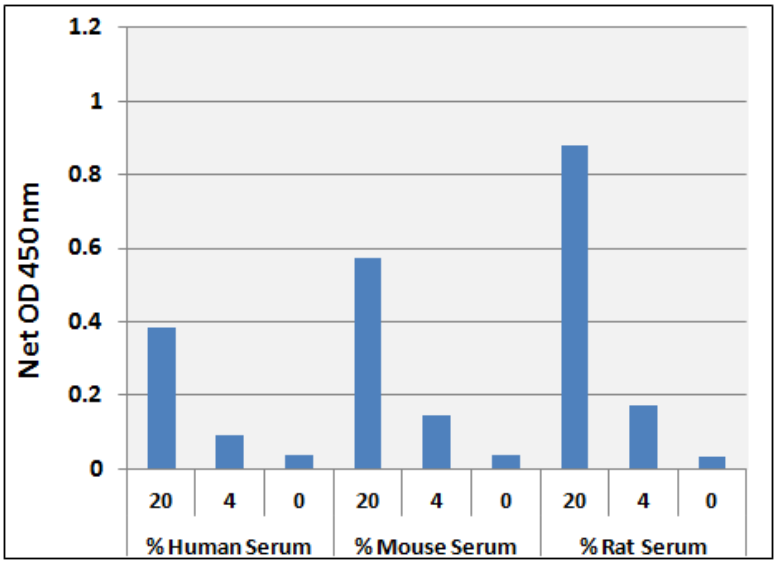

Figure 3. Detection of Glutamate in Serum. Human, mouse, or rat serum was assayed according to the kit protocol.

Calculation of Results.

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without GDH (-GDH) from the sample well values containing enzyme (+GDH) to obtain the difference. The absorbance difference is due to the enzyme GDH activity:

$$\Delta A = A_{(+GDH)} - A_{(-GDH)}$$

5. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of glutamate present in the sample. Only use values within the range of the standard curve.

References

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2. Jinap S and Hajeb P (2010) Appetite **55**: 1-10.
3. McEntee W.J. and Crook T.H. (1993) *Psychopharm (Berl)* **111**: 391-401.
4. Koochekpour S., Majumdar S., Azadaftari G., Attwood K., Scioneaux R., Subramani D., Manhardt C., Lorusso G.D., Willard S.S., Thompson H., Shourideh M., Rezaei K., Sartor O., Mohler J.L., and Vessella R.L. (2012) *Human Cancer Biol.* **18**: 5888-5901.
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Recent Product Citation

Mannion, A. et al. (2019). Gamma-glutamyltranspeptidase expression by Helicobacter saguini, an enterohepatic Helicobacter species isolated from cotton top tamarins with chronic colitis. *Cell Microbiol*. **21**(3): e12968. doi: 10.1111/cmi.12968.

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