Amplite[™] Fluorimetric Glutamic Acid Assay Kit **Red Fluorescence**

| Ordering Information | Storage Conditions | Instrument Platform |
|------------------------------------|--------------------------------------------|---------------------------------|
| Product Number: 10054 (200 assays) | Keep in freezer Avoid exposure to light | Fluorescence microplate readers |

Introduction

Glutamic acid is one of the 20 proteinogenic amino acids. The carboxylate anions and salts of glutamic acid are known as glutamates. Glutamate is an important neurotransmitter which plays a key role in long-term potentiation and is important for learning and memory. Glutamic acid is the precursor of GABA but has somewhat the opposite function; it might play a role in the normal function of the heart and the prostate. As one of the few nutrients that crosses the blood-brain barrier, glutamic acid is used in the treatment of diseases such as depression, ADD and ADHD, fatigue, alcoholism, epilepsy, muscular dystrophy, mental retardation, and schizophrenia.

The AmpliteTM Fluorimetric Glutamic Acid Assay Kit provides a quick and sensitive method for the measurement of glutamic acid in various biological samples. In the assay, the coupled enzyme system catalyzes the reaction between L-glutamic acid and NADP to produce NADPH, which is specifically recognized by NADPH sensor and recycled back to NADP. A red flurescence product is produced during the reaction. The signal can be read by either a fluorescence microplate reader at Ex/Em = 530-570 nm/590-600 nm (optimal Ex/Em = 540 nm/590 nm) or an absorbance microplate reader at 576 ± 5 nm. With our AmpliteTM Fluorimetric Glutamic Acid Kit, we have detected as little as 1µM glutamic acid in a 100 µL reaction volume. The assay is robust, and can be readily adapted for a wide variety of applications that require the measurement of glutamic acid.

| Kit Key Features | | | |
|--------------------|--------------------------------------------------------------------------|--|--|
| Broad Application: | Can be used for quantifying glutamic acid in various biological samples. | | |
| Sensitive: | Detect as low as 1 µM of glutamic acid in solution. | | |
| Continuous: | Easily adapted to automation without a separation step. | | |
| Convenient: | Formulated to have minimal hands-on time. No wash is required. | | |
| Non-Radioactive: | No special requirements for waste treatment. | | |

Kit Components

| Components | Amount |
|------------------------------|-------------------------------|
| Component A: Enzyme Mixture | 1 bottle (lyophilized powder) |
| Component B: Assay Buffer | 1 bottle (10 mL) |
| Component C: NADP | 1 vial |
| Component D: Glutamic Acid | 1 vial |
| Component E: Dilution Buffer | 1 bottle (10 mL) |

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare glutamic acid assay mixture (50 µL) → Add glutamic acid standards or test samples (50 µL) → Incubate at room temperature for 30 minutes – 2 hours → Monitor fluorescence increase at Ex/Em = 540/590 nm

Note: Thaw all the kit components at room temperature before starting the experiment.

1. Prepare NADP stock solution (200X):

Add 100 μ L of Dilution Buffer (Component E) into the vial of NADP (Component C) to make 200X NADP stock solution.

Note: The unused NADP *stock solution should be divided into single use aliquots and stored at -20°C*.

2. Prepare glutamic acid stock solution:

Add 200 µL of Dilution Buffer (Component E) into the vial of Glutamic Acid (Component D) to make 100 mM glutamic acid stock solution.

Note: The unused glutamic acid stock solution should be divided into single use aliquots and stored at -20 °C.

3. Prepare glutamic acid assay mixture:

- 3.1 Add 10 mL of Assay Buffer (Component B) into the bottle of Enzyme Mixture (Component A).
- 3.2 Add 50 μL 200X NADP stock solution (from Step 1) into the Enzyme Mixture bottle (from Step 3.1), and mix them well.

Note: This glutamic acid assay mixture is enough for two 96-well plates. The unused glutamic acid assay mixture should be divided into single use aliquots and stored at -20°C.

4. Prepare serially diluted glutamic acid standards (0 to 1 mM):

4.1 Add 10 μL of glutamic acid stock solution (from Step 2) into 990 μL Dilution Buffer (Component E) to generate 1 mM glutamic acid standard solution.

Note: Diluted glutamic acid standard solution is unstable. Use within 4 hours.

- 4.2 Take 200 μL of 1 mM glutamic acid standard solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 μM serially diluted glutamic acid standards.
- 4.3 Add serially diluted glutamic acid standards and glutamic acid containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

| BL | BL | TS | TS | | | | |
|------|------|----|----|------|--|--|--|
| GLU1 | GLU1 | | | | | | |
| GLU2 | GLU2 | | | | | | |
| GLU3 | GLU3 | | | | | | |
| GLU4 | GLU4 | | | | | | |
| GLU5 | GLU5 | | | | | | |
| GLU6 | GLU6 | | | | | | |
| GLU7 | GLU7 | | | | | | |

 Table 1. Layout of glutamic acid standards and test samples in a solid black 96-well microplate

Note: GLU= Glutamic Acid Standards, BL=Blank Control, TS=Test Samples.

| Table 2. | Reagent | composition | for each well | |
|----------|---------|-------------|---------------|--|
| | | | | |

| Glutamic Acid Standards | Blank Control | Test Sample |
|--------------------------|-------------------------|-------------|
| Serial Dilutions*: 50 µL | Dilution Buffer : 50 µL | 50 μL |

*Note: Add the serially diluted glutamic acid standards from 1 μ M to 1 mM into wells from GLU1 to GLU7 in duplicate.

5. Run glutamic acid assay:

- 4.1 Add 50 μL of glutamic acid assay mixture (from Step 3) into each well of glutamic acid standard, blank control, and test samples (see Step 4.3) to make the total glutamic acid assay volume of 100 μL/well. Note: For a 384-well plate, add 25 μL of sample and 25 μL of glutamic acid assay mixture into each well.
- 4.2 Incubate the reaction at room temperature for 30 minutes to 2 hours, protected from light.
- 4.3 Monitor the fluorescence increase by using a fluorescence plate reader at Ex/Em = 530-570/590-600 nm (optimal Ex/Em = 540/590 nm).

Note: The contents of the plate can also be transferred to a white clear bottom plate and read by absorbance microplate reader at the wavelength of 576 \pm 5 nm. The absorption detection has lower sensitivity compared to the fluorescence reading.

Data Analysis

The fluorescence in blank wells (with the dilution buffer only) is used as a control, and is subtracted from the values for those wells with the glutamic acid reaction. A glutamic acid standard curve is shown in Figure 1. *Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.*



Figure 1. Glutamic acid dose response was measured with AmpliteTM Fluorimetric Glutamic Acid Assay Kit in a black 96-well plate using a Gemini (Molecular Devices) microplate reader. As low as 1 μ M glutamic acid was detected with 1 hour incubation.

References

- 1. Arai S, Shibata H, Sakai M, Ninomiya H, Iwata N, Ozaki N, Fukumaki Y. (2009) Association analysis of the glutamic acid decarboxylase 2 and the glutamine synthetase genes (GAD2, GLUL) with schizophrenia. Psychiatr Genet, 19, 6.
- AuCoin DP, Sutherland MD, Percival AL, Lyons CR, Lovchik JA, Kozel TR. (2009) Rapid detection of the poly-gamma-D-glutamic acid capsular antigen of Bacillus anthracis by latex agglutination. Diagn Microbiol Infect Dis, 64, 229.
- Blanc F, Ruppert E, Kleitz C, Valenti MP, Cretin B, Humbel RL, Honnorat J, Namer IJ, Hirsch E, Manning L, de Seze J. (2009) Acute limbic encephalitis and glutamic acid decarboxylase antibodies: a reality? J Neurol Sci, 287, 69.
- 4. Boyer AE, Quinn CP, Hoffmaster AR, Kozel TR, Saile E, Marston CK, Percival A, Plikaytis BD, Woolfitt AR, Gallegos M, Sabourin P, McWilliams LG, Pirkle JL, Barr JR. (2009) Kinetics of lethal factor and poly-D-glutamic acid antigenemia during inhalation anthrax in rhesus macaques. Infect Immun, 77, 3432.

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