Amplite™ Fluorimetric Glutamic Acid Assay Kit *Red Fluorescence*

Catalog number: 10054
Unit size: 200 Tests

Component	Storage	Amount
Component A: Enzyme Mix	Freeze (<-15 °C), Minimize light exposure	1 bottle (lyophilized powder)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component C: NADP	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: Glutamic Acid	Freeze (<-15 °C), Minimize light exposure	1 vial
Component E: Dilution Buffer	Freeze (<-15 °C), Minimize light exposure	1 bottle (10 mL)

OVERVIEW

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 Amplite™ 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 our 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 or an absorbance microplate reader. With our Amplite™ Fluorimetric Glutamic Acid Kit, we have detected as little as 10 µM 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.

AT A GLANCE

Protocol summary

1. Prepare Glutamic Acid working solution (50 µL)
2. Add Glutamic Acid standards and/or test samples (50 µL)
3. Incubate at room temperature for 30 minutes - 2 hours
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm (Cutoff = 570 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

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

2. Glutamic Acid standard solution (100 mM):
Add 200 µL of Dilution Buffer (Component E) into the vial of Glutamic Acid (Component D) to make 100mM Glutamic Acid standard solution.

PREPARATION OF STANDARD SOLUTION

Glutamic Acid standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10054

Take 100 mM Glutamic Acid standard solution and perform 1:100 in Dilution Buffer (Component E) to make 1000 µM Glutamic Acid standard solution (SD7). Take 1000 µM Glutamic Acid standard solution (SD7) and perform 1:3 serial dilutions to get serially diluted Glutamic Acid standards (SD6 - SD1) with Dilution Buffer (Component E).

PREPARATION OF WORKING SOLUTION

1. Add 10 mL of Assay Buffer (Component B) into the bottle of Enzyme Mix (Component A).

2. Add 50 µL 200X NADP stock solution into the Enzyme Mix bottle, and mix well to make Glutamic Acid working solution.

Note This Glutamic Acid working solution is enough for two 96-well plates. It is unstable at room temperature, and should be used promptly within 2 hours and avoid exposure to light.

Note Alternatively, one can make a 50X of Enzyme Mix stock solution by adding 200 µL of H2O into the bottle of Enzyme Mix (Component A), and then prepare the Glutamic Acid working solution by mixing the stock solution with Assay Buffer (Component B) and 200X NADP stock solution proportionally.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Glutamic Acid standards and test samples in a solid black 96-well microplate. SD = Glutamic Acid Standard, BL = Blank Control, TS = Test Sample.

BL	BL	TS	TS
SD1	SD1	...	...
SD2	SD2	...	...
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

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Table 2. Reagent composition for each well

Well	Volume	Reagent
SD1-SD7	50 µL	Serial Dilution (1 to 1000 µM)
BL	50 µL	Dilution Buffer (Component E)
TS	50 µL	Sample

1. Prepare Glutamic Acid standards (SD), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of Glutamic Acid working solution into each well of Glutamic Acid standard, blank control, and test samples to make the total Glutamic Acid assay volume of 100 µL/well. For a 384-well plate, add 25 µL of Glutamic Acid working solution into each well intead, for the total volume of 50 µL/well.
3. Incubate the reaction at room temperature for 30 minutes to 2 hours, protected from light.
4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 530 - 570 nm, Emission = 590 - 600 nm (optimal Ex/Em = 540/590 nm), Cutoff = 570 nm.

Note The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the absorbance ratio of ~570 nm to ~605 nm (A575nm/A605nm). The absorption detection has lower sensitivity compared to the fluorescence reading.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Glutamic Acid samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

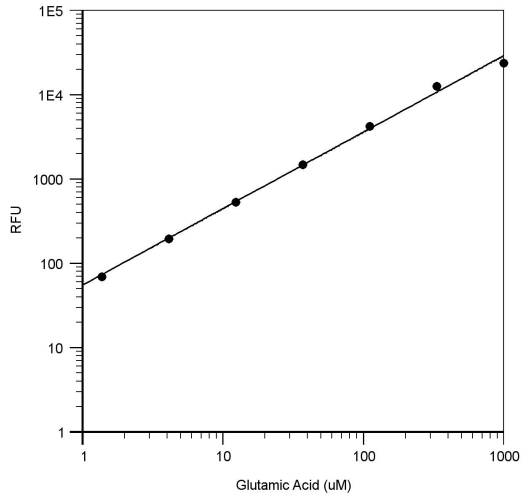

Figure 1. Glutamic acid dose response was measured with Amplite™ Glutamic Acid Assay Kit in a solid black 96-well plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER