

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 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 NADPH sensor and recycled back to NADP. A red fluorescence 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 Amplite™ 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.

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.

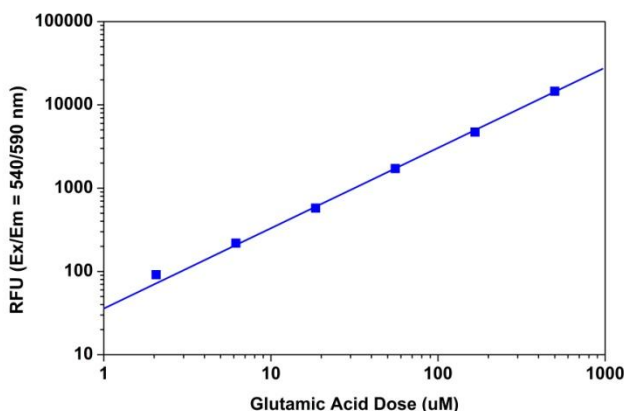


Figure 1. Glutamic acid dose response was measured with Amplitude™ Fluorimetric Glutamic Acid Assay Kit in a black 96-well plate using a Gemini (Molecular Devices) microplate reader. As low as 1 µM glutamic acid was detected with 1 hour incubation.

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.
2. 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.
3. 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.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.