Technical Manual

ChromaDazzle Glutamate Assay Kit

Catalogue Code: BA0114

Pack Size: 100 assays

Research Use Only

DESCRIPTION

Glutamate is an important chemical in general metabolism. It is also a crucial mammalian neurotransmitter that is believed to be involved in a number of neurological and psychiatric disorders such as lateral sclerosis, lathyrism, autism and Alzheimer's disease. Glutamate is also widely used as a flavor enhancer in the food industry.

Simple, direct and automation-ready procedures for measuring glutamate concentration are very desirable. The Assay Genie ChromaDazzle Glutamate Assay Kit is based on glutamate dehydrogenase catalyzed oxidation of glutamate, in which the formed NADH reduces a formazan (MTT) Reagent. The intensity of the product color, measured at 565 nm, is proportionate to the glutamate concentration in the sample.

APPLICATIONS

Direct Assays: glutamate in serum, plasma, tissue extracts and food extract samples.
Drug Discovery/Pharmacology: effects of drugs on glutamate levels.

KEY FEATURES

Sensitive and accurate. Detection limit of 50 µM, linearity up to 2.5 mM glutamate in 96-well plate assay.
Convenient. The procedure involves adding a single working reagent, and reading the optical density at time zero and at 30 min at room temperature. No 37°C heater is needed.
High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer: 10 mL **NAD Solution: 1 mL**
Enzyme Mix: 120 µL **MTT Solution: 1.5 mL**
Standard: 1 mL 100 mM Glutamate

Storage conditions. The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

1. *Calibration Curve*. Prepare 600 µL 2.5 mM Glutamate Premix by mixing 15 µL 100 mM Standard and 585 µL distilled water. Dilute standard as follows. Transfer 20 µL standards into wells of a clear bottom 96-well plate.

No	Premix + H_2O	Vol (µL)	Glutamate (mM)
1	100µL + 0µL	100	2.5
2	80µL + 20µL	100	2.0
3	60µL + 40µL	100	1.5
4	40µL + 60µL	100	1.0
5	30µL + 70µL	100	0.75
6	20µL + 80µL	100	0.5
7	10µL + 90µL	100	0.25
8	0µL + 100µL	100	0

Samples: add 20 µL sample per well in separate wells. *IMPORTANT*: Serum and tissue extract samples require a sample blank.

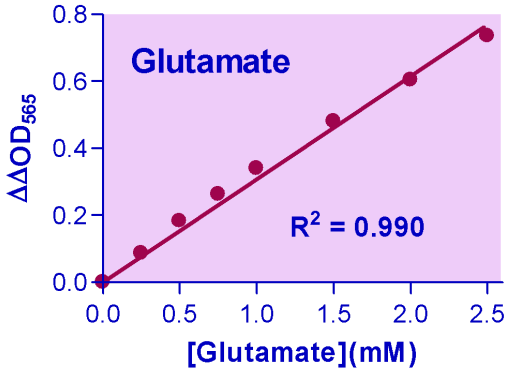
2. *Reagent Preparation*. Spin the Enzyme Mix tube briefly before pipetting. For each well of reaction, prepare Working Reagent by mixing 60 µL Assay Buffer, 1 µL Enzyme Mix, 5 µL NAD and 14 µL MTT. Fresh reconstitution is recommended. *Where a sample blank in required, prepare a Blank Working Reagent by mixing 60 µL Assay Buffer, 5 µL NAD and 14 µL MTT* **(i.e. No Enzyme Mix).**
3. *Reaction*. Add 80 µL Working Reagent (or Blank Working Reagent where appropriate) per reaction well quickly. Tap plate to mix briefly and thoroughly.
4. Read optical density (OD0) for time "zero" at 565 nm (520-600nm) and OD30 after a 30-min incubation at room temperature.
5. *Calculation.* Subtract OD0 from OD30 for the standard and sample wells. Next, subtract the ΔOD_{water} (Std 8) from each $\Delta OD_{standard}$ and ΔOD_{sample} to obtain the $\Delta\Delta$ODs. *(Where a sample blank was required, subtract the ΔOD_{blank} from ΔOD_{sample} to obtain the $\Delta\Delta OD_{sample}$.)* Plot the $\Delta\Delta OD_{standard}$'s and use this standard curve to convert the $\Delta\Delta OD_{sample}$ values to sample glutamate concentration.

$$[\text{Glutamate}] = \frac{\Delta\Delta OD_{SAMPLE}}{\text{Slope}} \text{ (mM)}$$

Note: If the sample $\Delta\Delta$OD values are higher than the $\Delta\Delta$OD value for the 2.5 mM glutamate standard, dilute sample in distilled water and repeat this assay. Multiply the results by the dilution factor.

Conversions: 1 mM glutamate = 14.5 mg/dL.

Standard Curve in 96-well plate assay



MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

GENERAL CONSIDERATIONS

1. This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of multi-channel pipettor is recommended.
2. The following substances interfere and should be avoided in sample preparation: EDTA (>0.5 mM), ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).

LITERATURE

1. Perez-de la Mora, M, et al (1989). A Glutamate Dehydrogenase-Based Method for the Assay of L-Glutamic Acid: Formation of Pyridine Nucleotide Fluorescent Derivitives. Anal. Biochem. 180: 248-252.

2. Matsumura, H. and Miyachi S (1980). Cycling assay for nicotinamide adenine dinucleotides. Methods Enzymol. 69: 465-470.
3. Graham, LT and Aprison, MH (1966). Fluorometric determination of aspartate, glutamate, and gamma-aminobutyrate in nerve tissue using enzymic methods. Anal. Biochem. 15: 487-497.

Contact Details

Dublin, Ireland

Email: info@assaygenie.com

Web: www.assaygenie.com

Technical Support: Techsupport@assaygenie.com