**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. BioAssay Systems’ EnzyChrom™ 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
- Working Reagent: 50 mL

**MATERIALS REQUIRED, BUT NOT PROVIDED**


**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.

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 is required, prepare a Blank Working Reagents 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 (OD₅₆₅) for time “zero” at 565 nm (520-600nm) and OD₅₆₅ after a 30-min incubation at room temperature.

5. Calculation. Subtract OD₅₆₅ from OD₅₆₅ for the standard and sample wells. Next, subtract the ∆OD₅₆₅ (Std 8) from each ∆OD₅₆₅ and ∆OD₅₆₅ to obtain the ∆∆OD₅₆₅. (Where a sample blank was required, subtract the ∆OD₅₆₅ from ∆OD₅₆₅ to obtain the ∆∆OD₅₆₅.) Plot the ∆∆OD₅₆₅ values and use this standard curve to convert the ∆∆OD₅₆₅ values to sample glutamate concentration.

[Glutamate] = \( \frac{\Delta\Delta OD_{565}}{\text{Slope}} \) (mM)

Note: If the sample ∆∆OD values are higher than the ∆∆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.

**LITERATURE**

