L-Glutamate Assay kit (Cat #: A-115)

**COMPONENTS:**
- L-Glutamate Assay Solution: 10 ml (for 200 assays); store at -80°C in aliquots
- 8 mM L-Glutamate Standard: 1 ml; store at -80°C

**PRODUCT DESCRIPTION:** Glutamate serves as an intermediate in transamination and deamination reactions, allowing nitrogen from amino acids to be excreted. It is also a critical neurotransmitter involved in learning and memory. The glutamate assay is based on the reduction of INT in a NADH-coupled enzymatic reaction to formazan, which exhibits an absorption maximum at 492 nm. The assay can detect L-glutamate in the range of 20 μM – 2 mM. Cell/tissue/plasma/serum samples should be deproteinized prior to assay. The assay solution is stable for several years if stored and handled properly.

**PROTOCOL:**

**Sample Preparation:**
Cell/tissue/serum/plasma samples can be extracted by TCA prior to assay. Please follow detailed sample extraction protocol at [http://www.bmrservice.com/SupplementTCA.html](http://www.bmrservice.com/SupplementTCA.html). Note that TCA is highly corrosive and Ether is highly flammable. Please contact us or visit the product webpage for MSDS information.

**Reagent thawing:**
Keep thawed Glutamate Assay Solution and Glutamate standard on ice. It is important to minimize the time that the reagents are thawed. Freeze reagents immediately after use.

**Glutamate standards:**
First dilute the 8 mM Glutamate standard 10-fold with dH₂O to obtain 0.8 mM (800 μM) Glutamate, e.g. 90 μl dH₂O + 10 μl 8 mM Glutamate. Perform serial 1:1 dilutions with dH₂O to obtain 400 μM, 200 μM and 100 μM Glutamate standards.

**Glutamate Assay:**
1. Add 20 μl Glutamate standards and samples to a 96-well microplate. Add 20 μl dH₂O to a control well as blank.
2. Gently agitate thawed Glutamate Assay Solution before pipetting. Reaction is initiated by addition of 50 μl Glutamate Assay Solution to the control, Glutamate standard and sample wells. Mix contents by gentle but thorough agitation for 30 sec. Cover plate and incubate at 37°C for 60 min.
3. Stop reaction by adding 50 μl 3% acetic acid (not provided) per well followed by gentle but thorough agitation. Eliminate any air bubbles present in the wells prior to measurement. Measure absorbance at 492 nm using a microplate reader.
4. Subtract the dH₂O blank reading from Glutamate standard and sample readings. Use the subtracted readings for plotting and calculation.
5. Plot Glutamate standards vs. their respective O.D.₄₉₂ nm. Generate a trendline equation on chart. Calculate sample Glutamate concentration using the derived equation (y = sample O.D.₄₉₂ nm; x = sample Glutamate concentration). A new plot must be generated for each assay.

**ADDITIONAL INFORMATION**
- Glutamate Assay Solution contains the organic solvent DMSO (9% v/v) and iodonitrotetrazolium violet (2 mg/ml). Please contact us or visit the product webpage for MSDS information.
- Sample may need to be diluted with dH₂O to obtain assay linearity. Multiply the result by the dilution factor where applicable.
- A 3% acetic acid solution needs to be prepared for reaction termination.