BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Glutamate Assay kit (Cat #: A-115)

COMPONENTS: Glutamate Assay Solution: 10 ml (for 200 wells); **store in aliquots at -70°C after the first thawing**
10 mM L-Glutamate: 0.2 ml, store at -70°C
PEG Solution: 5 ml; store at 4°C **(viscous; pipette solution with a cut tip)**

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 L-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 solution is stable for several years if stored and handled properly.

PROTOCOLS

Preparation of serum/plasma and cell culture medium: These samples are deproteinized by PEG precipitation. Mix 50 µl of each sample with 50 µl PEG Solution in a 1.5-ml microtube (PEG solution should be pipetted slowly using a cut tip). Vigorously vortex tube for at least ~30 sec to ensure thorough mixing. Keep tube on ice for 30 min. Centrifuge solution in a microfuge at ~13,000 rpm for 5 min at 4°C. Harvest supernatant and store at -20°C. Note that the sample has been diluted 2-fold.

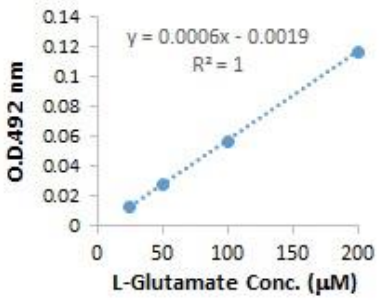
Preparation of tissue/cell samples: Cell and tissue samples are deproteinized by PEG precipitation. Please follow the extraction protocol at http://www.bmrservice.com/SupplementPEG.html. Alternatively, the samples can be deproteinized by TCA precipitation (http://www.bmrservice.com/SupplementTCA.html), which is recommended for analysis of nucleotides. Some TCA-treated samples may be diluted with dH_2O prior to assay to achieve optimal result.

L-Glutamate standard:

Dilute the 10 mM Glutamate standard 50-fold with dH_2O to 200 µM, e.g. 490 µl dH_2O + 10 µl 10 mM Glutamate. Perform additional 1:1 dilution with dH_2O to generate 100 µM, 50 µM and 25 µM Glutamate. Store diluted standards at -20°C.

ASSAY

1. Thaw Glutamate Assay Solution quickly and keep solution on ice shielded from light during assay. Note: do not over thaw the solution, which can cause the solution to turn reddish and lose sensitivity.
2. Add 20 µl of each glutamate standard and deproteinized sample to a plain (uncoated) 96-well plate.
3. Gently agitate Glutamate Assay Solution before first pipetting. Reaction is initiated by addition of 50 µl Glutamate Assay Solution to each glutamate standard and sample well. Mix contents by gentle but thorough agitation for 10 sec. Cover and incubate plate in a 37°C humidified incubator for 60 min. Note: Do not use CO_2 incubator.
4. Optional: Stop reaction by adding 50 µl 3% acetic acid (not provided in the kit) per well followed by gentle but thorough agitation. Eliminate air bubbles present in wells prior to absorbance measurement.
5. Measure O.D. at 492 nm using a plate reader. Note that O.D. will be inflated if turbidity appears in wells. Samples can be diluted with dH_2O prior to assay to eliminate turbidity.
6. Plot glutamate standards vs. $O.D._{492\ nm}$. Generate a trend line equation on chart. Calculate sample glutamate concentration using the derived equation (x = sample glutamate concentration in µM; y = $O.D._{492\ nm}$). A new plot must be generated for each assay.



ADDITIONAL INFORMATION

Glutamate Assay Solution contains the organic solvent DMSO and iodonitrotetrazolium violet. Please contact us or visit the product webpage for MSDS information.