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

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Glutamine/Glutamate Assay Kit (Cat #: A-133)

COMPONENTS: Glutamine Conversion Buffer- 20 ml, store at 4ºC
Glutaminase- 25 µl, store at 4ºC **(gently vortex vial to disperse enzyme precipitates prior to pipetting)**
Glutamate Assay Solution- 5 ml (for 100 wells); store at -70°C
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: The assay is based on (1) conversion of L-glutamine to L-glutamate by glutaminase, and (2) glutamate deamination/oxidation by glutamate dehydrogenase in the presence of NAD^+. The NADH formed can be quantified at 492 nm using a microplate format. Reagents are stable for at least one year if stored and handled properly.

PROTOCOLS

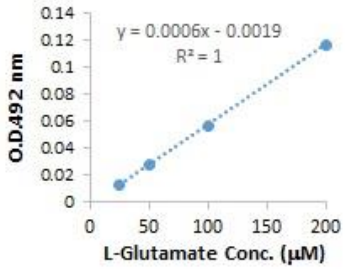
Preparation of serum/plasma and cell culture medium: These samples are deproteinized by PEG precipitation. Mix 25 µl of each sample with 25 µl PEG Solution in a 1.5-ml microtube (PEG solution should be pipetted slowly using a cut tip). Vigorously vortex tube for at least ~30 sec to ensure thorough mixing. 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.

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.

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. Prepare 1 ml of conversion solution by mixing 1 µl Glutaminase and 1 ml Glutamine Conversion Buffer. Mix well and keep solution on ice. Discard unused portion. Note: Gently vortex Glutaminase vial prior to pipetting.
2. Set up a **control well (for glutamate)** and **conversion well (for glutamate + glutamine)** for each sample. Pipet 5 µl of each deproteinized sample to a plain (uncoated) 96-well plate in duplicate.
3. Add 15 µl dH_2O to each well of the control set. Add 15 µl freshly prepared conversion solution to each well of the conversion set. Gently agitate plate for 10 sec. Cover and place plate in a 37°C humidified incubator for 60 min (do not use CO_2 incubator). Glutamine present in the conversion wells will be converted to glutamate.
4. Remove plate from incubator. Add 20 µl of each glutamate standard to a set of empty wells. Add 50 µl ice-cold Glutamate Assay Solution to each control well, conversion well, and glutamate standard well.
5. Mix contents by gentle but thorough agitation for 10 sec. Cover and place plate in a 37°C humidified incubator for 60 min (do not use CO_2 incubator). This step measures the amount of glutamate present in wells.
6. Stop reaction by adding 50 µl 3% acetic acid (not provided in the kit) per well followed by gentle agitation. Eliminate air bubbles present in wells if necessary. Measure O.D. at 492 nm using a plate reader.
7. Plot glutamate standards vs. $O.D._{492\ nm}$. Generate a trend line equation on chart. Calculate glutamate concentration of control wells and conversion wells for each sample using the derived equation (x = glutamate concentration in µM; y = $O.D._{492\ nm}$). A new plot must be generated for each assay. **Multiply the glutamate concentration of each sample well by 4**.
8. **Sample [glutamine] = $[glutamate]_{conversion\ well}$ - $[glutamate]_{control\ well}$**



ADDITIONAL INFORMATION

- Glutamate Assay Solution contains the organic solvent DMSO and iodonitrotetrazolium violet. Please contact us or visit the product webpage for MSDS information. A solution of 3% acetic acid needs to be prepared for termination of glutamate assay.