BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

COMPONENTS: Glutaminase Buffer- 5 ml, store at 4ºC
Glutaminase- 20 µl, store at 4ºC **(gently vortex vial to disperse enzyme solution prior to pipetting)**
Glutamate Assay Solution- 5 ml (for 100 wells); store at -80°C (**shield solution from light**)
10 mM Glutamate- 0.2 ml, store at -20°C or -80°C
PEG Solution- 3 ml, store at room temperature **(agitate vial to redissolve contents upon arrival)**

PRODUCT DESCRIPTION: The Glutamine/Glutamate Assay Kit is based on conversion of glutamine to glutamate by glutaminase followed by the glutamate dehydrogenase reaction in the presence of NAD^+. Reagents are stable for at least one year if stored and handled properly.

PROTOCOLS

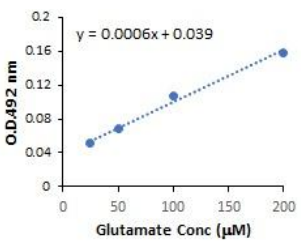
Preparation of serum/plasma and cell culture medium: These samples are deproteinized by PEG precipitation. Mix 50 µl of each sample with 50 µl PEG Solution in a 1.5-ml microtube (PEG solution should be pipetted slowly using a cut tip). Shake tube vigorously followed by vortexing to ensure thorough mixing. Keep tube on ice for 3 min. Centrifuge solution in a microfuge at ~14,000 rpm for 3 min. 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 https://bmrservice.com/deproteination-by-peg. Alternatively, the samples can be deproteinized by TCA precipitation (https://bmrservice.com/deproteination-by-tca). Deproteinized samples may be diluted with dH_2O prior to assay to achieve optimal results.

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 1:1 serial dilution with dH_2O to generate 100 µM, 50 µM and 25 µM Glutamate. Store diluted standards at -20°C.

ASSAY:

1. Prepare conversion solution by mixing 1 µl Glutaminase and 0.1 ml ice-cold Glutaminase Buffer. Mix well and keep solution on ice. Use the conversion solution immediately. Discard unused portion.
2. Use a plain (uncoated) 96-well plate for the assay. Designate a set of glutamate standard wells and add 20 µl of each glutamate standard to the well (25-200 µM).
3. Proceed to add 20 µl of each deproteinized sample to duplicate wells (for sample glutamate well and sample conversion well). The sample conversion well measures glutamate AND glutamine.
4. Add 10 µl dH_2O to each glutamate standard well and each sample glutamate well. Add 10 µl freshly prepared conversion solution to each sample conversion well. Briefly mix contents by gentle agitation. Cover and place plate in a 37°C humidified incubator for 60 min. *Do not use CO_2 incubator.*
5. Remove plate from incubator. Add 50 µl ice-cold Glutamate Assay Solution to each well. Briefly mix contents by gentle agitation. Cover and place plate in a 37°C humidified incubator for 90 min.
6. Measure O.D. at 492 nm using a plate reader.
7. Plot glutamate standards vs. $O.D._{492nm}$. Generate a trend line equation on chart (see graph below). Calculate glutamate concentration of the sample glutamate well and sample conversion well for each sample using the derived equation (x = glutamate concentration in µM; y = $O.D._{492nm}$). A new plot must be generated for each assay.
8. Sample glutamate concentration is derived from the sample glutamate well. Sample glutamine concentration = $[glutamate]_{sample\ conversion\ well}$ - $[glutamate]_{sample\ glutamate\ well}$



ADDITIONAL INFORMATION

- Agitate the PEG Solution vial in warm water to re-dissolve contents upon arrival.
- Glutamate Assay Solution contains iodonitrotetrazolium chloride and the organic solvent DMSO. Please contact us or visit the product webpage for material SDS information.