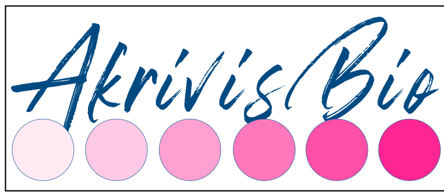


MA-0133

Glutamate Assay

(100 wells, Colorimetric, OD 450 nm, Store at -20°C)

Introduction:

Glutamate, a non-essential amino acid, plays several important metabolic roles in the body. As a neurotransmitter, it facilitates communication between nerve cells, contributing to brain function and cognition. Glutamate is also a key molecule in protein synthesis, aiding in the production of other amino acids. It also serves as a precursor for glutathione, a potent antioxidant that helps protect cells from oxidative damage. Glutamate is also involved in energy metabolism, as it can be converted into α-ketoglutarate, a crucial intermediate in the citric acid cycle. AkrivisBio's Glutamate assay is a simple, sensitive means of assessing glutamate content in a variety of tissues with an ability to detect less than 0.2 nmoles per well.

Assay Principle:

1 - Glutamate is oxidatively converted to α-ketoglutarate and NH_3 by glutamate dehydrogenase with the conversion of NAD to NADH.
2 - NADH is utilized to convert a tetrazolium to a highly colored formazan with λ_{max} of 450 nm.

Assay Components:

Assay Buffer	25 ml	WM	MA-0133-A
Glutamate Dehydrogenase	lyophil	Green	MA-0133-B
NAD/Tetrazolium Mix	lyophil	Red	MA-0133-C
Glutamate Standard	100 µl	Yellow	MA-0133-D

Storage and Handling:

Store the kit at -20°C. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening..
Assay Buffer: Ready to use as supplied. Store at 4°C
Glutamate Dehydrogenase: Reconstitute with 220 µl Assay Buffer. Store at -20°C. Keep on ice while in use. If the assay is to be used repeatedly over a period of time, aliquot the enzyme into convenient portions and store at -20°C
NAD/Tetrazolium Mix: Reconstitute with 220 µl DI water.
Glutamate Standard (40 mM): Ready to use as supplied.

Assay Protocol:

1. Standard Curve: Transfer 10 µl of the Glutamate standard to 990 µl Assay Buffer giving an 0.4 mM solution. Transfer 0 – 5 – 10 – 15 – 20 - 25 µl of the standard to a series of wells of a 96-well plate in duplicate to generate 0, 2, 4, 6, 8, 10 nmol/well. Adjust all well volumes to 50 µl with Assay buffer.

2. Sample Preparation: Homogenize tissue (10 mg) or cells (1×10^6) in 100 µl Assay Buffer. Centrifuge at 16,000 X g, 5 minutes to remove particulates. Transfer the clear supernatant to a fresh tube.. Transfer 2 – 50 µl samples to a 96 well plate. Adjust all wells to 50 µl with Assay Buffer. Prepare a parallel sample well as the background control. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.
Note: Some samples give a high background. To deal with this eventuality, run samples in duplicate with one of the pair being used as a background control.

3. Initiate Reaction: Each reaction requires 100 µl of reaction mix. Prepare sufficient reaction mix for the total number of wells to be run:

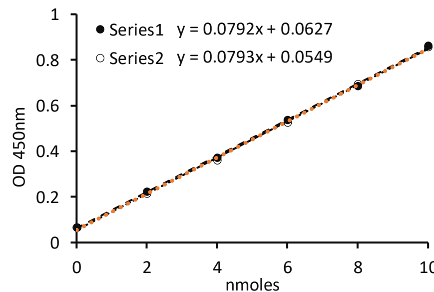
	Reaction Mix:	Background Control Mix
Assay Buffer	90 µl	92 µl
NAD/Tetrazolium Mix	2 µl	2 µl
Glutamate Dehydrogenase	2 µl	------

Add 100 µl of the Reaction Mix to each well containing the Glutamate Standard and test samples. Add 100 µl of background control mix to each background control well. Mix

4. Measurement: Monitor absorbance at 450 nm at 37°C for 30 minutes in a microplate reader.

5. Typical Results:

nmol Standard	Standard Raw Values (OD)		Background Corrected Values (OD)	
0	0.0656	0.0650	0	0
2	0.2241	0.2133	0.1585	0.1483
4	0.3732	0.3588	0.3076	0.2938
6	0.5381	0.5244	0.4725	0.4594
8	0.6869	0.6941	0.6213	0.6291
10	0.8632	0.8542	0.7976	0.7892



6. Calculation: Subtract the 0 standard reading from all standards. Plot the standard curve for the background corrected values. Determine the slope of the standard curve. The slope defines the OD/nmol obtained from the assay. Subtract background control well readings from each paired unknown sample. Divide the background corrected unknown values by the slope of the standard curve to determine the glutamate content of the sample wells. To convert those values to the glutamate content of the original samples:
A. nmoles glutamate in well / µl sample applied to well = nmoles glutamate/µl of sample applied to well
B. nmoles glutamate/µl of sample applied to well X total volume of sample applied to well = total nmoles glutamate in sample applied to well.
C. total nmoles glutamate in sample applied to well/ mg tissue (or # of cells, etc.) = nmoles/ mg (# of cells, etc)

FOR RESEARCH USE ONLY! Not to be used on humans