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Product Information

Glutamate Assay Kit

Catalog Number **MAK330**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Glutamate is an important chemical in general metabolism. It is also a crucial mammalian neurotransmitter that is believed to be involved in a number of neurological and psychiatric disorders such as lateral sclerosis, lathyrism, autism, and Alzheimer's disease. Glutamate is also widely used as a flavor enhancer in the food industry.

Simple, direct, and automation-ready procedures for measuring glutamate concentration are very desirable. The Glutamate Assay Kit is based on glutamate dehydrogenase catalyzed oxidation of glutamate, in which the formed NADH reduces a formazan (MTT) reagent. The intensity of the product color, measured at 565 nm, is proportionate to the glutamate concentration in the sample.

The Glutamate Assay Kit has a detection limit of 50 μM, linearity up to 2.5 mM glutamate in a 96 well plate assay.

Suitable for glutamate determination in serum, plasma, tissue extracts, and food extract samples. Also suitable in drug discovery/pharmacology for studying the effects of drugs on glutamate levels.

Components
The kit is sufficient for 100 colorimetric assays in 96 well plates.

Assay Buffer Catalog Number MAK330A	10 mL
NAD Solution Catalog Number MAK330B	1 mL
MTT Solution Catalog Number MAK330C	1.5 mL
Enzyme A Catalog Number MAK330D	120 μL
Enzyme B Catalog Number MAK330E	120 μL
Standard (100 mM Glutamate) Catalog Number MAK330F	1 mL

Reagents and Equipment Required but Not Provided.
- Pipetting devices and accessories (e.g., multichannel pipettor)
- Centrifuge tubes
- 96 well flat bottom plate. It is recommended to use clear plates for colorimetric assays
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on dry ice. Store all components at –20 °C upon receiving.

Procedure
Sample Preparation
Serum and tissue extract samples require a sample blank.

The following substances interfere with the assay and should be avoided in sample preparation: ascorbic acid, EDTA (>0.5 mM), sodium dodecyl sulfate (>0.2%), sodium azide, NP-40 (>1%) and TWEEN® 20 (>1%).

Standard Curve

Prepare 600 µL of 2.5 mM Glutamate Premix by mixing 15 µL of 100 mM Standard and 585 µL of ultrapure water. Dilute standard according to Table 1.

Table 1.
Preparation of Glutamate Standards

Tube	Glutamate Premix	Ultrapure Water	Glutamate (mM)
1	100 µL	0 µL	2.5
2	80 µL	20 µL	2.0
3	60 µL	40 µL	1.5
4	40 µL	60 µL	1.0
5	30 µL	70 µL	0.75
6	20 µL	80 µL	0.5
7	10 µL	90 µL	0.25
8	0 µL	100 µL	0

Reaction Mix

Note: Briefly centrifuge enzyme tubes before opening.

For each well of reaction, prepare Reaction Mix by mixing:

60 µL of Assay Buffer
1 µL of Enzyme A
1 µL of Enzyme B
5 µL of NAD
14 µL of MTT

Fresh preparation just prior to use is recommended.

Where a sample blank is required, prepare a Blank Reaction Mix by mixing:

60 µL of Assay Buffer
1 µL of Enzyme B
5 µL of NAD
14 µL of MTT
(No Enzyme A)

Assay Reaction

1. Transfer 20 µL of standards into separate wells of a clear, flatbottom 96 well plate.
2. Transfer 20 µL of each sample into separate wells.
3. Add 80 µL of Reaction Mix (or Blank Reaction Mix where appropriate) per reaction well quickly. Tap plate to mix briefly and thoroughly.
   Note: This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Reaction Mix to samples should be quick and mixing should be brief but thorough. Use of a multichannel pipettor is recommended. Assays can be executed at room temperature or 30 °C.
4. Immediately measure the initial absorbance at 565 nm (A_{565}).
5. Incubate plate for 30 minutes at room temperature.
6. Measure the final absorbance at 565 nm (A_{565}).

Results

Calculate the ΔA_{565} by subtracting the initial A_{565} from the final A_{565} for the standard and sample wells. Next, subtract the ΔA_{565} of Standard Tube 8 from each ΔA_{565} for all samples and standards to obtain the $\Delta(\Delta A_{565})$.

Where a sample blank was required, subtract the ΔA_{565} of the blank from the ΔA_{565} of the sample to obtain the sample $\Delta(\Delta A_{565})$. Plot the $\Delta(\Delta A_{565})$ of the standards and use this standard curve to convert the $\Delta(\Delta A_{565})$ sample values to sample glutamate concentration.

$$[\text{Glutamate, (mM)}] = \frac{\Delta(\Delta A_{565})\text{Sample}}{\text{Slope}}$$

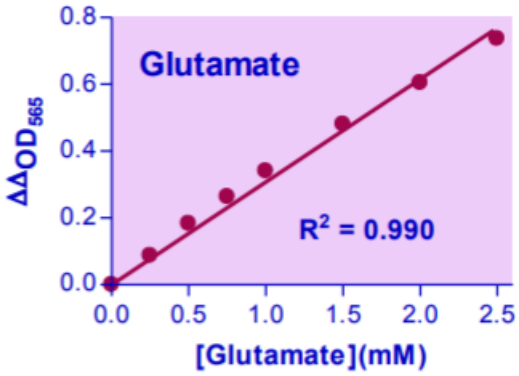
Note: If the sample A_{565} value is higher than A_{565} for the 2.5 mM glutamate standard, dilute sample in ultrapure water and repeat the assay. Multiply the results by the dilution factor.

Conversion

1 mM glutamate = 14.6 mg/dL

Figure 1.

Typical Standard Curve



References

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2. Matsumura, H., and Miyachi, S., Cycling assay for nicotinamide adenine dinucleotides. Methods Enzymol., **69**, 465-470 (1980).
3. Graham, L.T., and Aprison, M.H., Fluorometric determination of aspartate, glutamate, and γ--aminobutyrate in nerve tissue using enzymic methods. Anal. Biochem., **15**, 487-497 (1966).

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