Glutamate Assay (GLU)

Cat. No. 8338
100 Tests in 96-well plate

Introduction

Glutamate is a key molecule in cellular metabolism and an important mammalian neurotransmitter that is believed to be involved in a number of neurological diseases such as lateral sclerosis, lathyrism, autism and Alzheimer's disease. Glutamate is also widely used as a flavor enhancer in the food industry. This colorimetric assay is based on glutamate dehydrogenase catalyzed oxidation of glutamate, in which the formed NADH reduces a tetrazolium salt, MTT. The intensity of the product color, which exhibits maximum absorbance at 570 nm, is proportional to the glutamate concentration in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8338a	1	Assay buffer	10 mL	4°C
8338b	1	Glutamate standard	1 mL	4°C
8338c	1	NAD	0.4 mL	-20°C
8338d	1	Substrate	8 mg	-20°C
8338e	1	Substrate solvent	1.5 mL	-20°C
8338f	1	Enzyme	0.1 mL	-20°C

Product Use

This kit measures glutamate level of samples from cells, serum, plasma, tissue, and food extracts. It is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from Glutamate Assay of glutamate solutions with concentrations ranging from 0.02 to 1 mM show a linear relationship between OD_{570nm} and glutamate concentration (Figure 1).

Shipping and Stability

Shipped on dry ice and the kit is stable for one year when handled properly.

Procedure (96-well plate)

A. Preparation of glutamate standard

1. Add 1 µL of glutamate standard (8338b) to 99 µL of assay buffer (8338a) to make a 0.1 mL solution of 1 mM glutamate. Dilute standard as follows.

No	1mM(µL)	Assay buffer(µL)	Volume (µL)	Glutamate (mM)
1	10	0	10	1 mM
2	7.5	2.5	10	0.75 mM
3	5	5	10	0.5 mM
4	2.5	7.5	10	0.25 mM
5	1	9	10	0.1 mM
6	0.5	9.5	10	0.05 mM
7	0	10	10	0 mM

2. Transfer 10 µL each glutamate standard into wells of the 96-well flat bottom plate.

B. Preparation of substrate solution

1. Combine substrate (8338d) and substrate solvent (8338e) and mix thoroughly to make substrate solution.
2. Store prepared substrate solution at -20°C.

C. Preparation of test samples

1. Cells or tissues can be homogenized in 4 volumes of the assay buffer (8338a). Centrifuge the samples at 13,000 ×g for 10 minutes to remove insoluble material. The soluble fraction may be assayed directly.
2. Endogenous NADH from cell or tissue extracts generates background in the glutamate assay. As a control to remove the endogenous NADH background, the same amount of sample can be tested in the absence of enzyme (8338f).
3. Samples should be serial diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.

D. Working reagent preparation and measurements

1. For each well of reaction, prepare working reagent by mixing 70 µL assay buffer (8338a), 4 µL NAD (8338c), 15 µL of substrate solution (prepared in step B), and 1 µL enzyme (8338f). When a control is required to remove endogenous NADH background from a sample, prepare a control working reagent by mixing 71 µL assay buffer (8338a), 4 µL NAD (8338c) and 15 µL of substrate solution (prepared in step B).
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing glutamate standard,

samples, and blank. Incubate for 20 minutes at room temperature protected from light.
3. Read the absorbance at 570 nm with an ELISA plate reader.

E. Calculations

1. Subtract the OD_{570nm} value of the blank from the OD_{570nm} values obtained with all other standard and samples to get ΔOD_{570nm} value.
2. Based on the calibrated ΔOD_{570nm} of the glutamate standard, make a standard curve by plotting ΔOD_{570nm} as a function of glutamate concentration (See Figure 1 for a typical standard curve). Determine the equation and R^2 value of the trend line.
3. For samples requiring control without enzyme, subtract the ΔOD_{570nm} without enzyme value from the ΔOD_{570nm} with enzyme value and use this $\Delta\Delta OD_{570nm}$ value to determine the sample glutamate concentration from the standard curve.
4. Because the equation of the trend line of the standard curve is $y = Ax + B$, calculate the glutamate concentration of test samples as follows:

$$[glutamate] = \frac{\Delta\Delta OD_{570nm}}{A}$$

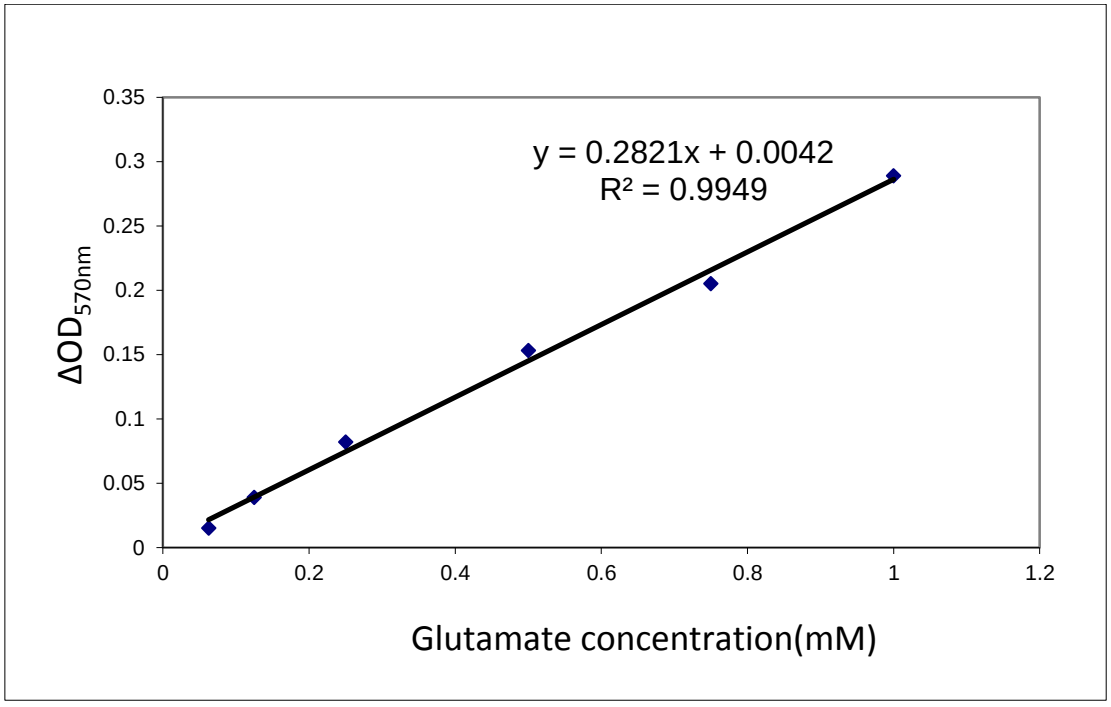

Figure1. A typical glutamate standard curve measured by ScienCell™ Glutamate Assay kit