# Glutamate Microplate Assay Kit

## Cat #: orb219861 (manual)

Detection and Quantification of Glutamate Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

***For research use only. Not for diagnostic or therapeutic procedures.***

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## INTRODUCTION

Glutamate, one of the two acidic proteinogenic amino acids, is also a key molecule in cellular metabolism. In humans, glutamate plays an important role both in amino acid degradation and disposal of excess or waste nitrogen. Glutamate is the most abundant swift excitatory neurotransmitter in the mammalian nervous system. It is believed to be involved in learning and memory and has appeared to be involved in diseases like amyotrophic lateral sclerosis, lathyrism, autism, some forms of mental retardation and Alzheimer's disease. Glutamic acid is also present in a wide variety of foods, and has been used as a flavor enhancer in food industry.

Glutamate Microplate Assay Kit is a sensitive assay for determining glutamate concentration in various samples. The glutamate reacts with the dye, and can be measured at a colorimetric readout at 570 nm.

## KIT COMPONENTS

| Component | Volume | Storage |
|---|---|---|
| 96-Well Microplate | 1 plate | |
| Assay Buffer | 30 ml x 4 | 4 °C |
| Dye Reagent A | Powder x 1 | 4 °C |
| Dye Reagent A Diluent | 7.5 ml x 1 | 4 °C |
| Dye Reagent B | 2.5 ml x 1 | 4 °C |
| Standard | Powder x 1 | 4 °C |
| Plate Adhesive Strips | 3 Strips | |
| Technical Manual | 1 Manual | |

**Note:**

**Standard:** add 1 ml Assay Buffer to dissolve before use, then add 0.1 ml into 0.9 ml Assay Buffer, the concentration will be 3 mmol/L, store at 4 °C for 1 month after reconstitution.

**Dye Reagent**: add 7.5 ml Dye Reagent A Diluent to Dye Reagent A dissolve before use, then add 2.5 ml Dye Reagent B, mix; store at 4 °C for 1 month after reconstitution.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

## SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for $5 \times 10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times) ; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Add 1 ml Assay buffer for 0.1 ml serum or plasma; mix; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

## ASSAY PROCEDURE

Add following reagents into the microplate:

| Reagent | Sample | Standard | Blank |
|---|---|---|---|
| Sample | 100 µl | -- | -- |
| Standard | -- | 100 µl | -- |
| Assay Buffer | -- | -- | 100 µl |
| Dye Reagent | 100 µl | 100 µl | 100 µl |
| Mix, keep at room temperature for 20 minutes, record absorbance measured at 570 nm. | | | |

**Note:**

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

## CALCULATION

1. According to the protein concentration of sample

$$\text{Glutamate (μmol/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 3 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the quantity of cells or bacteria

$$\text{Glutamate (μmol/10}^4\text{ cell)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay})$$

$$= 3 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

3. According to the weight of sample

$$\text{Glutamate (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 3 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

4. According to the volume of sample

$$\text{Glutamate (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times V / V_{Assay})$$

$$= 3 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V$$

$V_{Sample}$: the volume of the sample, 0.1 ml;

$V_{Standard}$: the volume of the standard, 0.1 ml;

$V_{Assay}$: the volume of Assay buffer, 1 ml;

$C_{Standard}$: the standard concentration, 3 mmol/L = 3 μmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

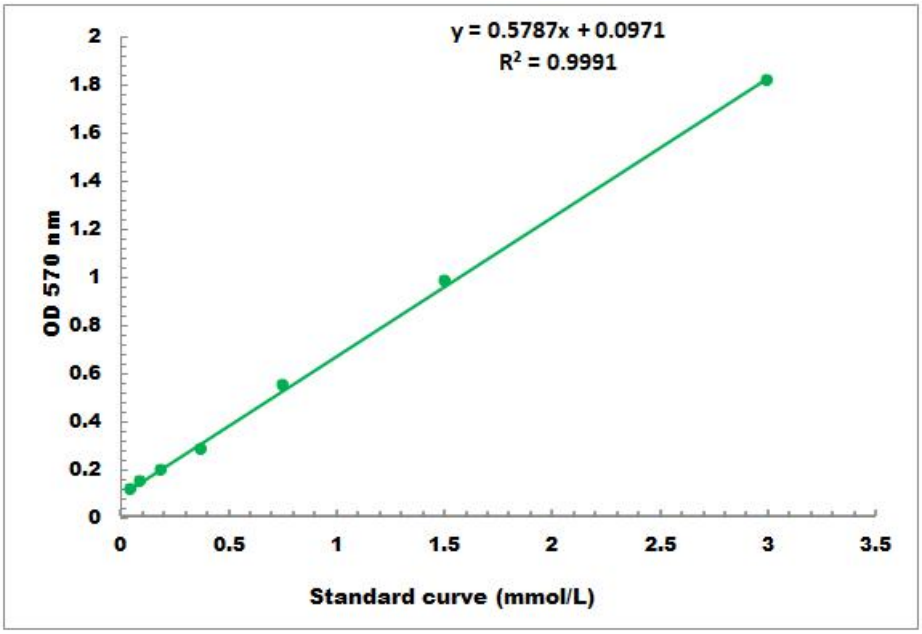
W: the weight of sample, g;

V: the volume of serum or plasma;

N: the quantity of cell or bacteria, $N \times 10^4$.

## TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.




Detection Range: 0.03 mmol/L - 3 mmol/L

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