Glutamate Microplate Assay Kit

Catalog # AS0005

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

Contact information:

Tel:+1 (301) 446-2499 Fax:+1 (301) 446-2413

Email:techcn@signalwayantibody.com Web:www.sabbiotech.com

I. 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.

The enzyme catalysed reaction products can be measured at a colorimetric readout at 570 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Dye Reagent	Powderx 1	4 °C
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Dye Reagent:add 10 ml distilled water heat to dissolve before use.

Standard:add 1 mlAssay Buffer to dissolve before use, then add 0.5 ml into 0.5 mlAssay Buffer. The concentration will be 500 μg/ml, store at 4 °C

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Convection oven

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

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

2.For tissue samples

Weigh0.1 g tissue, homogenize with 1mlAssay buffer on ice, centrifuged at 8000g 4°C for 10minutes, 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.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	100 µl	--	--
Standard	--	100 µl	--
Assay Buffer	--	--	100 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, put it into the convection oven,90 °C for 20 minutes, record absorbance measured at 570 nm.			

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{Glutamate}(\mu g/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 500 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the quantity of cells or bacteria

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

$$= 500 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

3. According to the weight of sample

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

$$= 500 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

4. According to the volume of serum or plasma

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

$$= 500 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V$$

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;

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

$C_{Standard}$: the standard concentration, 500 μg/ml;

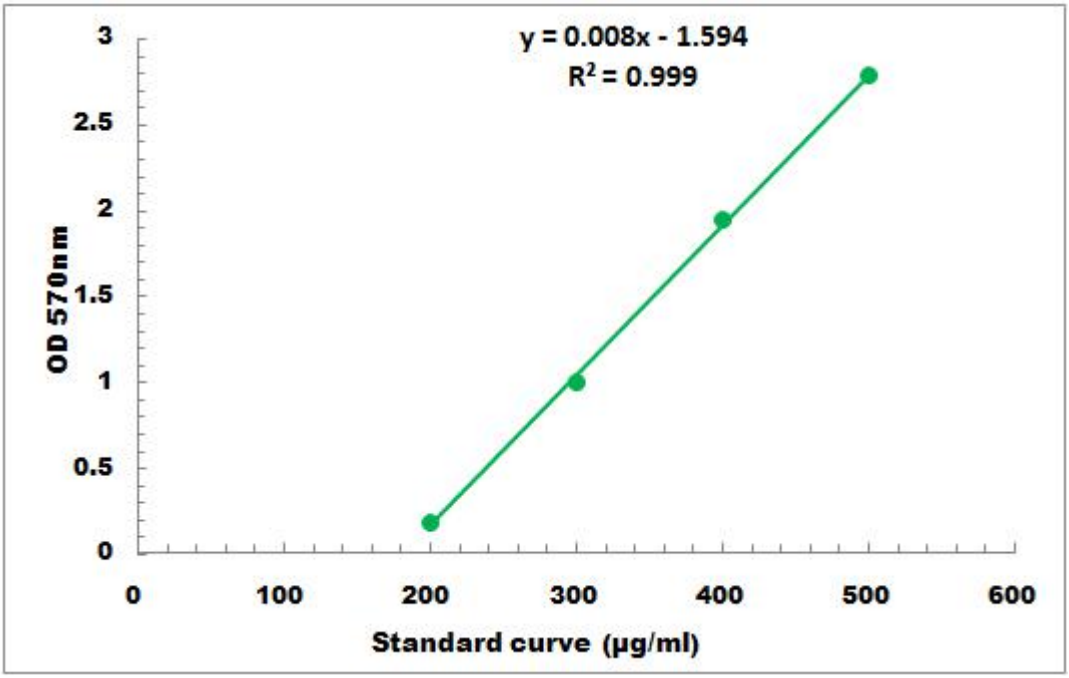
W: the weight of sample, g;

V: the volume of serum or plasma;

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

VII. TYPICAL DATA

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



Detection Range: 100 μg/ml - 500 μg/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES