Glutamine Synthetase Microplate Assay Kit

Catalog # AS0021

Detection and Quantification of Glutamine Synthetase Activity in Tissue extracts, Cell lysate Samples.

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

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

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I. INTRODUCTION

Glutamine Synthetase (GS) is mainly present in plants, is one of the key enzymes in vivo assimilation of ammonia.GS cancatalyze ammonium ions and glutamic acidto synthesiseglutamine Gln, not only can prevent excessive biological toxic ammonium ions, but alsoglutamine Gln is the main storage and transport in the form of ammonia.

In the presence of ATP and Mg^{2+}, GS cancatalyze ammonium ions and glutamic acidto synthesiseglutamine Gln; glutamine Gln further converted to gamma-glutamyl hydroxamic acid, under acidic conditions to form a red iron complexes; the complex has a maximum absorption peak at 540nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	8 mlx 1	4 °C
Substrate	Powder x 1	-20 °C
Dye Reagent	5 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate:add 3.5mldistilled waterinto the bottle to dissolve it absolutely before use, store at 4 °C.

Standard:add 1mldistilled water into the tube to dissolve before use, then add 0.25 ml into 0.75mldistilled water, mix, the concentration will be 5 mmol/L;store at 4 °C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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

Weighout 0.1 g tissue, homogenize with 1 mlAssay 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 liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Control	Standard	Blank
Reaction Buffer	80 µl	80 µl	--	--
Substrate	35µl	35µl	--	--
Distilled water	--	35µl	--	--
Sample	35µl	--	--	--
Mix, put it in the oven,37°C for 30minutes.				
Standard	--	--	150 µl	--
Distilled water	--	--	--	150 µl
Dye Reagent	50 µl	50 µl	50 µl	50 µl
Mix, record absorbance measured at 540 nm.				

VI. CALCULATION

Unit Definition: one unitis defined as the enzyme products 1nmol ofthegamma-glutamyl hydroxamic acidper minute.

1. According to the protein concentration of sample

$GS\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$

$= 714.3 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

2. According to the weight of sample

$GS\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$

$= 714.3 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$

$C_{Standard}$: the standard concentration, 5 mmol/L = 5000nmol/ml;

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

W: the weight of sample, g;

$V_{Standard}$: the volume of standard, 0.15 ml;

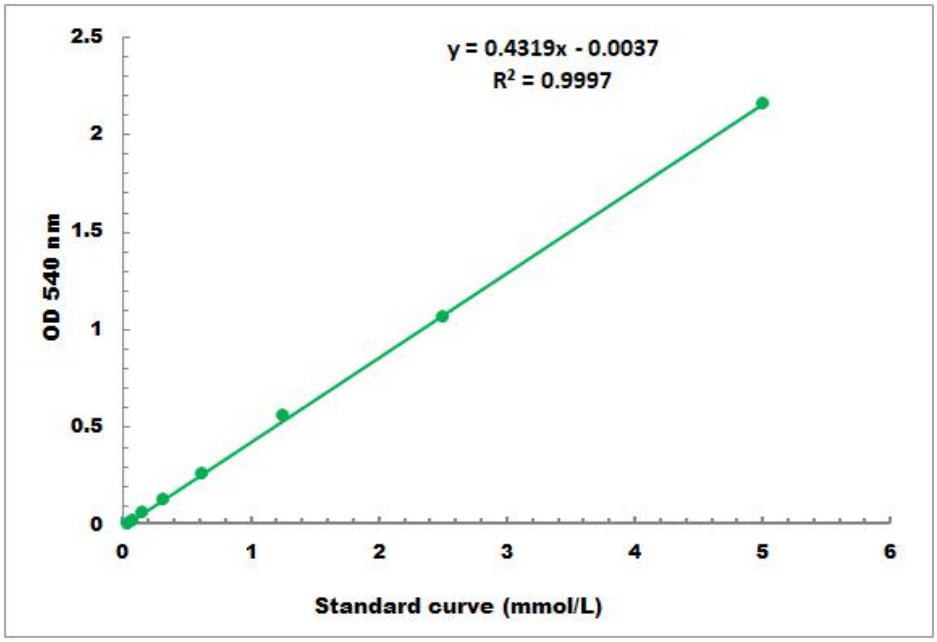
V_{Sample}: the volume of sample, 0.035 ml;

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

T: the reaction time, 30 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.05 mmol/L - 5 mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES