# Glutamine Synthetase

# Microplate Assay Kit

# User Manual

## Catalog # CAK1021

(Version 1.2G)

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

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

## I. INTRODUCTION

Glutamine Synthetase (GS) is mainly present in plants, is one of the key enzyme in vivo assimilation of ammonia. GS can catalyze ammonium ions and glutamic acid to synthesise glutamine Gln, not only can prevent excessive biological toxic ammonium ions, but also glutamine Gln is the main storage and transport in the form of ammonia.

In the presence of ATP and $Mg^{2+}$, GS can catalyze ammonium ions and glutamic acid to synthesise glutamine 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 ml x 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 3.5 ml distilled water into the bottle to dissolve it absolutely before use, store at 4 °C.

**Standard**: add 1 ml distilled water into the tube to dissolve before use, then add 0.25 ml into 0.75 ml distilled 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, multi-channel 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 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 out 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 liquid samples

Detect directly.

## V. ASSAY PROCEDURE

Add following reagents into the microplate:

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

**Note:**

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

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

**Unit Definition:** one unit is defined as the enzyme products 1 nmol of the gamma-glutamyl hydroxamic acid per 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 = 5000 nmol/ml;

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

W: the weight of sample, g;

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

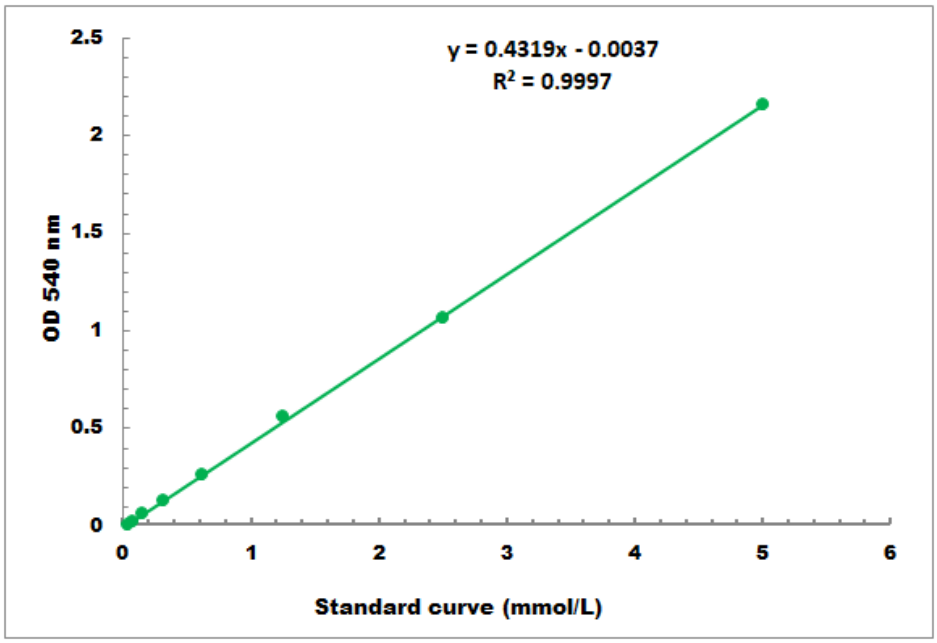
$V_{Sample}$: the volume of sample, 0.035 ml;

$V_{Assay}$: 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.cohesionbio.com or contact us at techsupport@cohesionbio.com

## IX. NOTES