Glutamine Synthetase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1021

(Version 2.2J)

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 assimilations of ammonia.GS cancatalyze ammonium ions and glutamic acidto synthesizeglutamine Gln, not only can prevent excessive biological toxic ammonium ions, but alsoglutamine Gln is the main storage and transport in the form of ammonia.

Glutamine Synthetase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining Glutamine Synthetase activity in various samples. Glutamine Synthetase activity is determined by NADH decomposition rate. The reaction products can be measured at a colorimetric readout at 450 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Substrate I	8 mlx 1	4 °C
Substrate II	Powder x 1	-20 °C, keep in dark
Substrate II Diluent	1 mlx 1	4 °C
Coenzyme	Powder x 1	-20 °C, keep in dark
Enzyme	Powder x 1	-20 °C
Enzyme Diluent	5 mlx 1	4 °C
Dye ReagentA	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 mlx 1	4 °C, keep in dark
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening. Dissolvein 1 mldistilled water to generate 2mmol/Lstock standard solution. Store stock solution at -20 °C for 2 weeks.Add 0.1 ml stock solution into 0.4 mldistilled water, the concentration will be 400 µmol/L as top standardsolution. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25µmol/L.

Substrate II: Briefly centrifuge prior to opening.Add1ml Substrate II Diluent before use. Store at 4°C for 2-3 days or -20 °C for 2 months.

Coenzyme: Briefly centrifuge prior to opening. Add1 ml distilled water to dissolve before use.Store at -20 °C for 1 month.

Enzyme: Briefly centrifuge prior to opening. Add1 ml Enzyme Diluent to dissolve before use.Store at -80 °C for 1 month.

Positive Control: Briefly centrifuge prior to opening. Add0.3 ml Enzyme Diluent to dissolve before use.Store at -80 °C for 1 month.

Dye Reagent A: Briefly centrifuge prior to opening. Add 7 ml distilled water to dissolve before use, mix, store at 4°C for 1 monthafter reconstitution.

V. 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%, sonicate 3s, interval 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.

VI. ASSAY PROCEDURE

Warm all regents to room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Substrate I	80 µl	80 µl	80 µl	--	--
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	20 µl	120 µl
Positive Control	--	--	10 µl	--	--
Substrate II	10 µl	10 µl	10 µl	--	--
Enzyme	10 µl	10 µl	10 µl		
Coenzyme	10 µl	10 µl	10 µl		
Mix andincubate at 37°Cfor 15 minutes					
Standard	--	--	--	100 µl	--
Dye Reagent A	70 µl	70 µl	70 µl	70 µl	70 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl	10 µl
Mix andincubate at room temperature for 2 minutes, record absorbance measured at 450 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background

control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of Glutamine Synthetaseis defined as the enzyme reduces 1 μmolof NADH per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Control}-OD_{Sample}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1According to the quantity of cells

$$\text{Activity} = \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Control}-OD_{Sample})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample}/ V_{Assay}) \times T}(U/10^4)$$

2.2 According to the weight of sample

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

2.3 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Control}-OD_{Sample})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}\times T}(U/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

N: the quantity of total cell sample, 10^4

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

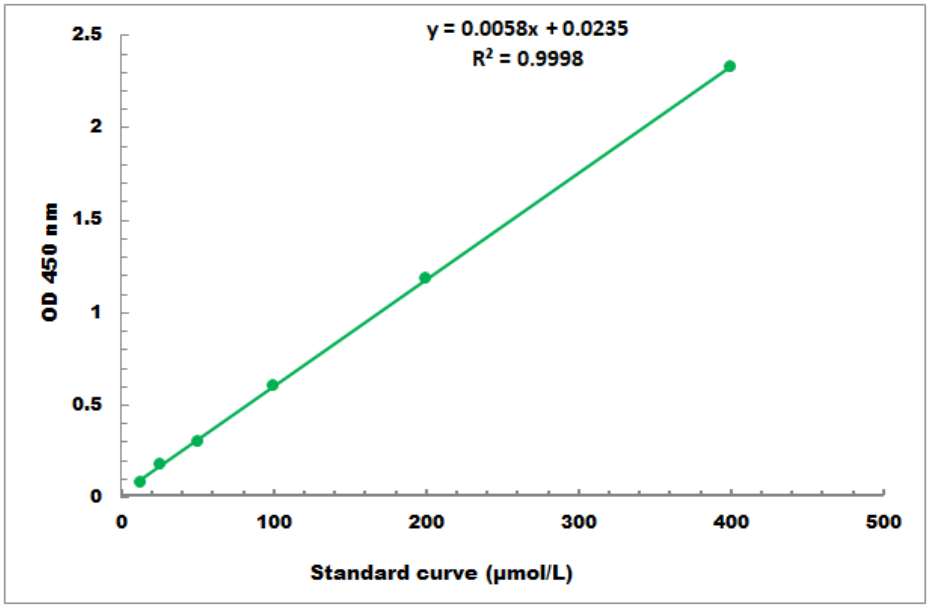
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

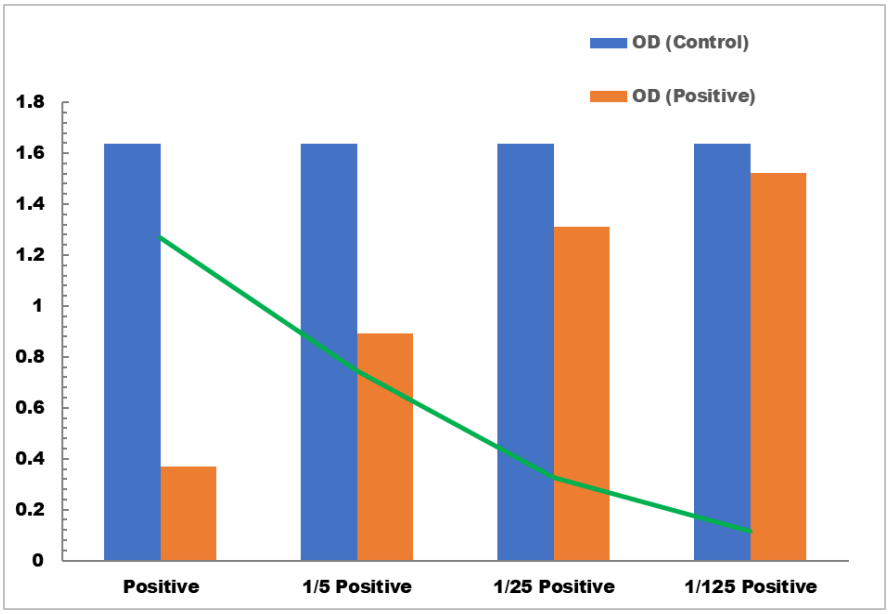
T: the enzyme reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 4μmol/L - 400 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration