Glutamine Synthetase Assay Kit

Catalog No.: abx097987

Size: 96 tests

Storage: Store all components in the dark at -20°C.

Application: For the quantification of glutamine synthetase (GS) activity in serum, plasma, tissue homogenates, and cell lysates.

Introduction

Glutamine synthetase (GS) is an ATP-dependent enzyme that catalyzes the formation of glutamine from glutamate and ammonium ions. Abbexa's Glutamine Synthetase Assay Kit is a quick, convenient, and sensitive method for the quantification of GS activity. Glutamine Synthetase in samples catalyzes the production of glutamine, which reacts with the Chromogenic Reagent to produce a colored compound with an absorbance maximum at 570 nm. The intensity of the color is proportional to the activity of GS, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 40 ml
3. Chromogenic Substrate: 1.8 ml
4. GS Enzyme Reagent: 3 vials
5. Acceleration Reagent: 3 vials
6. Catalyst: 3 vials
7. Standard Solution (5 mmol/L): 1 ml
8. Chromogenic Reagent: 0.3 ml
9. Stabilizer: 1.8 ml
10. Plate Sealer: 2

Materials Required But Not Provided

1. Microplate reader (570 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer
9. Incubator (37°C)

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Collect approximately 0.02 g of tissue and wash with pre-chilled PBS. Homogenize tissue sample in 180 µl of normal saline (0.9% NaCl) with a Dounce homogenizer at 4°C. Centrifuge the homogenate at 10,000 × g at 4°C for 10 min to remove insoluble material. Collect the supernatant and keep on ice. The protein concentration in the supernatant should be determined separately **(abx097193)**.
- **Cell lysates:** Collect 1 × 10^6 cells into a centrifuge tube and wash with PBS. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl of normal saline (0.9% NaCl) per 1 × 10^6 cells, then sonicate in an ice water bath. Centrifuge at 10,000 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately **(abx097193)**.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat Kidney Tissue Homogenate	4-10
10% Rat Liver Tissue Homogenate	5-20
10% Rat Spleen Tissue Homogenate	2-10
10% Rat Heart Tissue Homogenate	2-10
10% Rat Lung Tissue Homogenate	2-10
10% Rat Brain Tissue Homogenate	5-20
10% Mouse Liver Tissue Homogenate	5-20
10% Mouse Brain Tissue Homogenate	2-10
Rat serum	1
Rat plasma	1
Human serum	1
Pig serum	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Enzyme Working Solution:** Dissolve one vial of GS Enzyme Reagent with 200 µl of double distilled water. Mix thoroughly to dissolve. Use immediately, or store at -20°C in the dark for up to 7 days.
- **Acceleration Reagent Working Solution:** Dissolve one vial of Acceleration Reagent with 0.5 ml of buffer solution. Mix thoroughly to dissolve. Use immediately, or store at -20°C in the dark for up to 2 days.
- **Catalyst Working Solution:** Dissolve one vial of Catalyst with 1 ml of double distilled water. Mix thoroughly to dissolve. Use immediately, or store at -20°C in the dark for up to 2 days.
- **Reaction Working Solution:** Prepare 150 µl of Reaction Working Solution per well. The ratio of reagents is as follows:

Reagent	Enzyme Working Solution	Acceleration Reagent Working Solution	Stabilizer	Catalyst Working Solution	Buffer Solution
Ratio	1	1	1	2	29

The Reaction Working Solution should be prepared fresh before use and used within 4 hours.

- **Chromogenic Reagent Working Solution:** Prepare 40 µl of Chromogenic Working Solution per well. The Chromogenic Reagent should be diluted in Buffer Solution at a ratio of 1:19 (e.g. add 5 µl of Chromogenic Reagent to 95 µl of Buffer Solution to prepare 100 µl of Chromogenic Reagent Working Solution). Prepare fresh before use and use within 4 hours.
- **0.5 mmol/L Standard Solution:** Dilute 5 mmol/L Standard Solution with double distilled water at a 1:9 ratio. Use immediately, or store at -20°C for up to 7 days in the dark.

3. Preparation of standard curve

After preparation of fresh 0.5 mmol/L Working Standard Solution, carry out a serial dilution as follows:

Concentration (mmol/L)	0	0.1	0.15	0.2	0.3	0.4	0.45	0.5
0.5 mmol/L Standard Solution	0	20	30	40	60	80	90	100
Double distilled water (µl)	100	80	70	60	40	20	10	0

The 0 mmol/L standard serves as the blank.

B. Assay Procedure

1. Set standard, sample and control wells on the microplate and record their positions. Each sample requires a sample well and a control well. *Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
2. Add 20 µl of prepared standards to the standard wells.
3. Add 20 µl of sample to the sample wells.
4. Add 20 µl of sample to the control wells.
5. Add 20 µl of Chromogenic Substrate to the sample and standard wells. Add 20 µl of Buffer Solution to the control wells.
6. Add 150 µl of Reaction Working Solution to each well.
7. Add 40 µl of Chromogenic Reagent Working Solution to each well.
8. Use a microplate shaker or gently tap the plate to mix.
9. Incubate at 37°C in the dark for 30 minutes.
10. Measure the OD of each well with a microplate reader at 570 nm.

C. Calculation of Results

Standard Curve

1. Average the OD readings of both wells for each standard concentration.
2. Subtract the mean OD value of the blank (0 mmol/L standard) from all standard readings. This is the absolute OD value.
3. Plot the standard curve by using the absolute OD value on the y-axis, and the concentration of standard on the x-axis.
4. Create the standard curve (y = ax + b) with graph software.

Calculations:

1. Serum and plasma samples

One unit (U) is defined as the amount of GS enzyme in 1 L of serum or plasma per minute required to produce 1 µmol of substrate at 37°C.

$$\text{GS activity (U/L)} = \frac{\Delta A_{570} - b}{a \times T} \times 1000 \times f$$

2. Tissue and cell lysate samples:

One unit (U) is defined as the amount of GS enzyme in 1 g of sample protein per minute required to produce 1 µmol of substrate at 37°C.

$$\text{GS activity (U/g protein)} = \frac{\Delta A_{570} - b}{a \times T \times C_P} \times 1000 \times f$$

Where:

ΔA_{570}	$\Delta A_{570} = OD_{sample} - OD_{control}$
T	Reaction time (min)
f	Dilution factor of sample before test
C_P	Concentration of protein in sample (g protein/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)

D. Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.