Glutamine (Gln) Assay Kit

Catalog No.: abx298914

Size: 96 tests

Detection Range: 0.036 µmol/ml – 2.0 µmol/ml

Sensitivity: 0.036 µmol/ml

Storage: Store at -20°C in the dark.

Application: For detection and quantification of GLN activity in serum, plasma, tissue homogenates, and other biological fluids.

Introduction

Abbexa's GLN Assay Kit is a quick, convenient, and sensitive method for measuring and calculating GLN content. Glutamine is hydrolyzed by Glutaminase into Glutamic Acid. Glutamic acid is further converted to α-ketoglutarate and ammonia by glutamate dehydrogenase, while concurrently NAD^+ is reduced to NADH. WST-8 dye accepts electrons from NADH to produce a yellow-colored product, which has an absorbance maximum at 450 nm. The intensity of the color is proportional to GLN content, which can then be calculated.

Kit components

1. 96-well microplate
2. Enzyme Reagent A: 2 vials
3. Enzyme Reagent B: 2 vials
4. Enzyme Diluent: 24 ml
5. Accelerator Reagent: 1 vial
6. Substrate: 2 vials
7. Detection Reagent: 2 × 1.5 ml
8. Standard: 1 vial
9. Buffer Solution: 6 ml
10. Plate Sealer: 2

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Double Distilled Water
3. Normal saline (0.9% NaCl)
4. Pipette and pipette tips
5. Incubator (37°C)
6. Vials/tubes
7. 50 kDa Ultrafiltration tubes
8. Centrifuge

Protocol

A. Preparation of samples and reagents

1. Samples

Analyze samples immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **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. Centrifuge samples with a 50 kDa ultrafiltration tube at 10,000 × g for 15 minutes, then collect the filtrate on ice and assay immediately.
- **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. Centrifuge samples with a 50 kDa ultrafiltration tube at 10,000 × g for 15 minutes, then collect the filtrate on ice and assay immediately.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml normal saline (0.9 % NaCl). Homogenize by hand in an ice water bath, using a mechanical homogenizer or by ultrasonication. Centrifuge the homogenate at 10,000 x g for 15 minutes, take the supernatant to a fresh tube. Centrifuge with a 50 kDa ultrafiltration tube at 10,000 x g for 15 minutes, then collect the filtrate on ice and assay immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Normal Saline (0.9 % NaCl), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat liver tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat spleen tissue homogenate	3 – 5
10% *Epipremnum aureum* tissue homogenate	3 – 5
Rat plasma	1
Mouse plasma	1
Mouse serum	1
Human serum	2 – 3

Notes:

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

2. Reagents

- **Enzyme Reagent A Stock Solution:** Dissolve a vial of Enzyme Reagent A with 200 µl Buffer Solution and store on ice to use immediately, or at -20ºC in the dark for up to 3 days.
- **Enzyme Reagent A Working Solution:** Dilute the **Enzyme Reagent A** Stock Solution with Buffer Solution to a 1:9 ratio. Use within 4 hours of preparation.
- **Enzyme Reagent B:** Dissolve each vial with 200 µl double distilled water and store on ice for immediate use, or at -20 ºC in the dark for up to 3 days.
- **Accelerator Reagent:** Dissolve a vial of Accelerator Reagent with 1 ml double distilled water for immediate use, or store at -20 ºC in the dark for up to 3 days.
- **Substrate Stock Solution:** Dissolve each vial with 0.5 ml double distilled water for immediate use, or store at -20 ºC in the dark for up to 3 days.**Substrate Working Solution:** Mix the stock Substrate Solution with Enzyme Diluent and Accelerator Reagent in a 1:18:1 ratio. Prepare the required volume just before use, and use within 12 hours.
- **Reaction Working Solution:** Mix Enzyme Reagent B solution, Substrate Solution, and Detection Reagent in a ratio of 8:1192:200 and store on ice in the dark. Reaction Working Solution must be used within 1 hour of preparation.
- **Standard Solution:** Prepare the 100 µmol/ml stock solution by dissolving the Standard in 1 ml normal saline (0.9% NaCl). The stock solution can be stored at -20 ºC in the dark for 3 days. **Working Solution:** Prepare the 2 µmol/ml working solution by diluting the stock solution with normal saline (0.9% NaCl) to a 1:49 ratio. Prepare just before use, and use within 24 hours.

B. Assay Procedure

1. Standard curve preparation: Label tubes with 0, 0.4, 0.6, 0.8, 1.2, 1.6, 1.8, and 2.0 µmol/ml. Dilute the 2 µmol/ml Standard working solution with Normal Saline (0.9%) to produce 200 µl volumes of these concentrations.

2 µmol/ml Standard Working Solution (µl)	0.9% Normal Saline (µl)	Standard Concentration (µmol/ml)
0	200	0
40	160	0.4
60	140	0.6
80	120	0.8
120	80	1.2
160	40	1.6
180	20	1.8
200	0	2.0

2. Add 30 µl Enzyme Reagent A working solution to standard and sample wells as appropriate.
3. Add 50 µl Standard Solution dilution series to standard wells. Mix thoroughly.
4. Add 50 µl sample to the sample wells. Mix thoroughly.
5. Seal with a plate sealer, and incubate at 37ºC in the dark for 20 minutes.
6. Add 140 µl Reaction Working Solution to each well. Mix thoroughly.
7. Measure absorbance at 450 nm. Record these OD values as A_1.
8. Seal with a plate sealer, and incubate at 37 ºC in the dark for 30 minutes.
9. Measure absorbance at 450 nm, record OD values as A_2.

C. Calculation of Results

The standard curve can be plotted as the ΔA of each standard solution (*y*) vs. the respective concentration of the standard solution (*x*). A linear fit is recommended for the standard curve ($y = ax + b$). The GLN concentration of the samples can be interpolated from the standard curve using the following formulae:

1. Calculation of ΔA values for Standard, Sample, and Blank:

$$\Delta A = A_2 - A_1$$

2. Serum and plasma samples:

$$Gln\ content\ (\mu mol/ml) = \frac{\Delta A_{450} - b}{a} \times f$$

3. Tissue homogenate samples:

$$Gln\ content\ (\mu mol/g\ wet\ weight) = \frac{\Delta A_{450} - b}{a} \times \frac{f \times V}{m}$$

where:

y	$\Delta A_{Standard} - \Delta A_{Blank}$
x	Concentration of the standard
a	Slope of the standard curve (linear fit)
b	Intercept of the standard curve
ΔA_{450}	$\Delta A_{Sample} - \Delta A_{Blank}$
m	Wet weight of tissue (g)
v	Volume of tissue homogenate (ml)
f	Sample dilution factor