Glutaminase Assay Kit

Catalog No.: abx298831

Size: 96 tests

Detection Range: 0.003 U/L – 18.0 U/L

Sensitivity: 0.003 U/L

Storage: Store all components at -20°C. Store Substrate B, Enzyme Reagent, Accelerating Reagent, Chromogenic Reagent, and the Standards in the dark.

Application: For detection and quantification of Glutaminase activity in serum, plasma, and tissue homogenates.

Introduction

Glutaminase is a metabolic enzyme located primarily in the mitochondria. It converts the amino acid glutamine to glutamate, releasing ammonia, which provides a useful mechanism by which hepatocytes might regulate the pH of their cytoplasm, and provides energy for cell growth more generally. Recently, Glutaminase has received increased attention in the field of oncology. Compared to healthy cells, cancer cells often demonstrate a strong reliance on alternative metabolic pathways to the Krebs cycle – a phenomenon known as the Warburg effect. The reaction mediated by Glutaminase becomes a key driver of growth in tumors, and so it has been identified as a potential therapeutic target.

Abbexa's Glutaminase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Glutaminase activity. In the presence of glutamine, Glutaminase produces glutamic acid, which can be further oxidized by the enzyme glutamic acid dehydrogenase, reducing NAD^+ to NADH in the process. NADH can convert the chemical WST-8 to a yellow product, with an absorbance maximum at 450 nm. The intensity of the color is proportional to the Glutaminase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Substrate A: 2 vials
3. Substrate B: 2 vials
4. Reagent Diluent: 4 ml
5. Enzyme Reagent: 2 vials
6. Buffer Solution: 20 ml
7. Accelerating Reagent: 1 vial
8. Chromogenic Reagent: 2 × 1.5 ml
9. Standard (50 mmol/L): 2 vials
10. Plate sealer: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. PBS (0.01 M, pH 7.4)
3. Double distilled water
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Incubator

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** and **Plasma:** Serum and plasma samples can be tested directly. If there is significant turbidity in the samples, centrifuge at 8000 × g for 10 minutes, then carefully take the upper liquid phase for detection.
- **Tissue Homogenates:** Carefully weigh out a section of tissue, and add into PBS (0.01 M, pH 7.4) in a ratio of 1:9 weight (g) to volume (ml) (i.e. for every gram of tissue, add 9 ml of PBS). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath. Centrifuge at 10,000 × g for 15 minutes, then carefully remove the supernatant for detection. Assay immediately, or aliquot and store at -20°C. The protein concentration of the supernatant should be determined separately If there is significant turbidity in the samples, centrifuge at 8000 × g for 10 minutes, then carefully take the upper liquid phase for detection.

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 PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	1
Human plasma	1 – 3
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat kidney tissue homogenate	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

- **Substrate A Working Solution:** Dissolve the powdered Substrate A in 5 ml Double distilled water. Mix fully. The prepared solution can be stored at -20°C for up to 3 days.
- **Substrate B Working Solution:** Dissolve the powdered Substrate B in 0.4 ml Reagent Diluent. Mix fully. The prepared solution can be stored at -20°C for up to 3 days in the dark. Avoid repeated freeze-thaw cycles.
- **Enzyme Reagent Working Solution:** Dissolve the powdered Enzyme Reagent in 200 µl Double distilled water, and keep on ice. The prepared solution can be stored at 4°C for up to 6 hours. Keep in the dark.
- **Accelerating Reagent Working Solution:** Dissolve the powdered Accelerating Reagent in 1 ml Double distilled water. Mix fully. The prepared solution can be stored at -20°C for up to 3 days in the dark.
- **Reaction Working Solution:** Mix the Enzyme Reagent Working Solution, Buffer Solution, Substrate B Working Solution, and Accelerating Reagent Working Solution in a ratio of 10 : 690 : 37 : 10. Mix fully, then keep on ice until use. Prepare just before use, and use within 1 hour.
- **50 mmol/L Standard Stock Solution:** Dissolve the powdered Standard in 1 ml Reagent Diluent. Mix fully. The prepared solution can be stored at 4°C for up to 3 days.
- **Standards:** Dilute the 50 mmol/L Standard Stock Solution 100-fold with Double distilled water to create a diluted 0.5 mmol/L stock Standard. Label 7 tubes with 0.50 mmol/L, 0.45 mmol/L, 0.40 mmol/L, 0.30 mmol/L, 0.20 mmol/L, 0.15 mmol/L, and 0.10 mmol/L. Add 200 µl, 180 µl, 160 µl, 120 µl, 80 µl, 60 µl, and 40 µl of diluted Standard (0.5 mmol/L) to the 0.5 mmol/L, 0.45 mmol/L, 0.40 mmol/L, 0.30 mmol/L, 0.20 mmol/L, 0.15 mmol/L, and 0.10 mmol/L tubes respectively, followed by 0 µl, 20 µl, 40 µl, 80 µl, 120 µl, 140 µl, and 160 µl of PBS (0.01 M, pH 7.4), to prepare Standard Dilutions with concentrations 0.50 mmol/L, 0.45 mmol/L, 0.40 mmol/L, 0.30 mmol/L, 0.20 mmol/L, 0.15 mmol/L, and 0.10 mmol/L. These volumes are summarized in the following table:

Standard Dilution (mmol/L)	0.50	0.45	0.40	0.30	0.20	0.15	0.10
0.5 mmol/L Standard (µl)	200	180	160	120	80	60	40
PBS (0.01 M, pH 7.4) (µl)	0	20	40	80	120	140	160

For the blank, or 0 mmol/L standard, use pure PBS. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use, except the Enzyme Reagent Working Solution and the Reaction Working Solution, which must be kept on ice until use.
- Keep the Enzyme Reagent Working Solution, Substrate B Working Solution, and Accelerating Reagent Working Solution in the dark before use.

B. Assay Procedure

1. Mark microcentrifuge tubes for each sample and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 20 µl of sample to each sample tube, and 20 µl of the same sample to the corresponding control tube.
3. Add 80 µl Substrate A Working Solution to each sample tube.
4. Add 80 µl of Double distilled water to each control tube.
5. Mix fully, then incubate all tubes at 37°C for 30 minutes.
6. Centrifuge all tubes at 8000 × g for 5 minutes, then carefully take the supernatant for analysis on the 96-well plate.
7. On the 96-well plate, mark positions for each sample, control, and control. *It is strongly recommended to test in duplicate.*
8. Add 50 µl of each Standard Dilution to the corresponding wells. *Avoid foaming or bubbles.*
9. Add 50 µl of sample supernatant to the corresponding wells.
10. Add 50 µl of control supernatant to the corresponding wells.
11. Add 140 µl of Reaction Working Solution to all wells.
12. Add 20 µl of Chromogenic Reagent to all wells. Perform this step quickly to minimize exposure of the Chromogenic Reagent to direct light.
13. Immediately seal the plate with a plate sealer, to keep the contents of the wells in the dark.
14. Mix fully, then incubate the plate at 37°C for 20 minutes.
15. Measure and record the OD of each well with a microplate reader at 450 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Glutaminase in each sample well can be derived with the following formulae:

1. Serum and plasma samples:

One unit of Glutaminase activity is defined as the amount required for 1 L of serum or plasma to produce 1 µmol glutamic acid per minute at 37°C.

$$\text{Glutaminase (U/L)} = \mathrm{F} \times 1000 \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b}) \times \mathrm{V_{Tube}}}{\mathrm{a} \times \mathrm{t} \times \mathrm{V_{Plate}}}$$

2. Tissue samples:

Glutaminase activity in tissue samples can be calculated according to total protein concentration (which must be assayed separately).

One unit of Glutaminase activity is defined as the amount required for 1 g of tissue protein to produce 1 μmol of glutamic acid per minute at 37°C.

$$\text{Glutaminase (U/g protein)} = \mathrm{F} \times 1000 \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b}) \times \mathrm{V_{Tube}}}{\mathrm{a} \times \mathrm{t} \times \mathrm{C_{Protein}} \times \mathrm{V_{Plate}}}$$

where:

$\mathrm{OD_{Sample}}$	OD value of the sample
$\mathrm{OD_{Control}}$	OD value of the corresponding control
$\mathrm{V_{Tube}}$	Volume of liquid in the sample tubes (100 μl)
$\mathrm{V_{Plate}}$	Volume of liquid in the microplate wells (50 μl)
$\mathrm{C_{Protein}}$	Concentration of protein in sample (g/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
t	Time of the enzymatic reaction (30 mins)
F	The dilution factor of sample