Gamma-glutamyl Transferase Assay Kit

Catalog No.: abx298893

Size: 96 tests

Detection Range: 0.88 U/L – 339.4 U/L

Sensitivity: 0.88 U/L

Storage: Store all components at 4°C in the dark.

Application: For detection and quantification of Gamma-glutamyl transferase activity in serum, plasma, and animal tissue homogenates.

Introduction

Abbexa's Gamma-glutamyl transferase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Gamma-glutamyl transferase activity. Gamma-glutamyl transferase catalyzes the transfer of gamma glutamyl groups from glutamyl p nitroaniline to N-glycyl glycine. This produces p-nitroaniline, which has an absorption maximum of 405 nm. The intensity of the color is proportional to the Gamma-glutamyl transferase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Substrate: 2 vials
3. Buffer Solution: 30 ml
4. Extraction Solution: 2 × 50 ml
5. Standard Diluent: 10 ml
6. p-Nitroaniline Standard (1 mmol/L): 1.5 ml
7. Plate sealer: 2

Materials required but not provided

1. Microplate reader (405 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Centrifuge and centrifuge tubes
6. Vortex mixer
7. Incubator
8. Timer
9. Mechanical homogenizer

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.
- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue. Wash the tissue with cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue, add into 180 µl of Extraction Solution and homogenize manually, using a mechanical homogenizer at 4°C . Centrifuge at 10,000 × g for 10 minutes. Carefully take the supernatant, keep on ice and assay immediately.

  Note: To calculate Gamma-glutamyl transferase activity in tissue homogenates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (**abx097193**).

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 Extraction Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Mouse serum	1
Human serum	1
Rat serum	1
Dog serum	1
Hose serum	1
Pig serum	1
Human plasma	1
Human hydrothorax	1
10% Mouse liver tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat lung 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.
- Avoid hemolytic or lipemic samples.

2. Reagents

- **1 mmol/L Standard and Standard Diluent**: Preheat to 37°C until clarified.
- **Substrate Working Solution:** Reconstitue1 vial of Substrate with 3 ml of Standard Diluent. Mix thoroughly until completely dissolved. Aliquot and unused Substrate Working Solution can be stored at 4°C for up to 7 days.
- **Reaction Working Solution:**. Prepare enough Reaction Working Solution for the wells tested. Mix Buffer Solution and Substrate Working Solution at a ratio of 4:1. For example, prepare 250 µl of Reaction Working Solution by mixing 200 µl of Buffer Solution and 50 µl of Substrate Working Solution. This solution should be prepared just before use.
- **Standards:** Label 7 tubes with 200 µmol/L, 400 µmol/L, 500 µmol/L, 600 µmol/L, 800 µmol/L, 900 µmol/L and 1000 µmol/L. Prepare these dilutions according to the volumes in the following table

Standard Dilution (µmol/L)	200	400	500	600	800	900	1000
1 mmol/L Standard (µl)	40	80	100	120	160	180	200
Standard Diluent (µl)	160	120	100	80	40	20	0

For the blank, use pure Standard Diluent. The volume of each standard will be 200 µl.

Note:

- Allow all prepared reagents to equilibrate to room temperature before use.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Assign and record well locations for each standard and sample. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 25 µl of double-distilled water to the standard wells.
3. Add 25 µl of each sample to the corresponding wells.
4. Add 50 µl of each Standard to the corresponding standard wells followed by 200 µl of Buffer solution.
5. Add 250 µl of Reaction Working Solution to the sample wells.
6. Mix well the contents by tapping the plate, or shaking with a microplate shaker, for at least 10 seconds.

7. Incubate the microplate at 37 °C for 1 minute.
8. Measure the OD of each well with a microplate reader at 405 nm. Record these values as A_1.
9. Incubate the microplate at 37 °C for 5 minutes and measure the OD of each well at 405 nm. Record these values as A_2. *If the target concentration in the sample is expected to be low, it is recommended to extend the incubation time to 15 minutes.*

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 Gamma-glutamyl transferase in each sample well can be derived with the following formulae:

1. Serum and plasma:

One unit of Gamma-glutamyl transferase activity is defined as the amount of 1 μmol of p-nitroaniline catalyzed by 1L of sample in 1 minute at 37°C.

$$\text{Gamma-glutamyl transferase (U/L)} = \frac{(\triangle A - b) \times V_{Substrate}}{a \times t \times V_{Sample}} \times F$$

2. Tissue homogenates:

One unit of Gamma-glutamyl transferase activity is defined as the amount of 1 μmol of p-nitroaniline catalyzed by 1g of tissue protein in 1 minute at 37°C.

$$\text{Gamma-glutamyl transferase (U/g protein)} = \frac{(\triangle A - b) \times V_{Substrate}}{a \times t \times V_{Sample} \times C_{Protein}} \times F$$

Where:

$\triangle A$	$A_2 - A_1$
$V_{Substrate}$	Volume of Substrate Working Solution (5.0×10^{-5} L)
V_{Sample}	Volume of sample added (2.5×10^{-5} L)
$C_{Protein}$	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 (5 mins)
F	The dilution factor of sample

Technical Support

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