γ-Glutamyl Transferase (GGT) Enzymatic Assay Kit Manual

Catalog #: 5601-01

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γ-Glutamyl Transferase (GGT) Enzymatic Assay Kit is intended for laboratory use only. This product is NOT for clinical diagnostic use.

GENERAL INFORMATION

Product Description

The *γ-Glutamyl Transferase (GGT) Enzymatic Assay Kit* is a plate-based colorimetric enzymatic assay for the determination of the γ-glutamyl transferase enzyme in serum samples. γ-glutamyl transferase (GGT) is a metabolic enzyme expressed primarily in the liver, kidneys and other organs. Organ damage, especially damage to the liver, causes the release of this enzyme into the blood. Elevation of GGT levels is often an indication of liver damage and has been associated with liver injury as well as pancreatic and myocardial disorders. GGT is also a very useful tool for preclinical investigation of experimental drug formulations and GGT levels are commonly used to monitor and attenuate the toxic effects of experimental drug formulations in rodents.

The kit uses a spectrophotometric, kinetic assay to detect changes in γ-glutamyl transferase levels directly from serum samples. The unique features of the kit are:

- High sensitivity and low detection limit (<10 U/L)
- A rapid (10 minutes), robust enzyme-based assay which does not require expensive instrumentation
- High reproducibility
- Only requires 10 μL of serum

Procedure Overview

The *γ-Glutamyl Transferase (GGT) Enzymatic Assay Kit* uses an enzymatic reaction to measure enzyme levels in serum. The assay measures the cleavage of a specific GGT substrate (γ-glutamyl-p-ntiroanilide) by the enzyme. The production of the p-nitroaniline (pNA) product, measured at 405 nm, is proportional to the level of GGT enzyme in the sample. The absorbance of each sample well at 405 nm is measured using a plate reader. The concentration of GGT in each sample is then directly determined from the change in absorbance at 405 nm within 10 minutes. Dilutions of the pNA Control, included in the kit, can be used to construct a standard curve to calibrate the assay and confirm assay linearity. This is described in more detail in Section, "Data Analysis."

Kit Contents, Storage and Shelf Life

The *γ-Glutamyl Transferase (GGT) Enzymatic Assay Kit* has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). The kit also contains enough material to construct two standard curves. Store the kit at 4°C. The shelf life of the kit is 12 months when properly stored. Once the GGT Reagent Mix is reconstituted the shelf life of the kit is 6 months when properly stored. For more details, see "Preparation of GGT Reagent Mix".

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	4ºC or room temp
GGT Reagent Mix	Bottle	4ºC
pNA Control	0.4 ml	4ºC
pNA Dilution Buffer	2 x 1.8 mL	4ºC

Required Materials Not Provided With the Kit

- Microtiter plate reader (*405 nm*)
- Centrifuge (*to prepare serum samples*)
- Deionized or distilled water
- 1.5 mL microfuge tubes
- Multichannel pipet or repeating pipettor (*Optional*)
- PBS

Sensitivity (Detection Limit)

Sample Type	**Detection Limit** (U/L)
Serum	7

Warnings and Precautions

XPRESSBIO strongly recommends that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor or XPRESSBIO at info@xpressbio.com.

- Do not use the kit past the expiration date.
- Try to maintain a laboratory temperature of (20–25°C/68–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
- Make sure you are using only distilled or deionized water since water quality is very important.
- When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
- Wear gloves when performing the procedure.

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SAMPLE PREPARATION

Serum

1. Carefully collect whole blood in a 1.5 mL microfuge tube or serum collection tube making sure to avoid hemolysis.
2. Incubate the blood sample at 37°C for 10 minutes.
3. Centrifuge sample at 10,000 rpm for 10 minutes.
4. Remove serum layer to a clean tube avoiding the “buffy coat” layer.
5. Store serum samples on ice or at 4°C prior to testing; do not freeze samples. Serum samples can be stored at 4°C for up to one week.

γ-Glutamyl Transferase (GGT) DETECTION PROTOCOL

Reagent Preparation

IMPORTANT: Make sure you read "Warnings and Precautions" section. ALL REAGENTS AND THE MICROTITER PLATE SHOULD BE BROUGHT UP TO ROOM TEMPERATURE BEFORE USE (30 MIN - 1 HOUR AT 20–25°C/68–77°F).

Preparation of GGT Reagent Mix

To reconstitute the GGT Reagent Mix, add exactly 27 mL of deionized or distilled water to the GGT Reagent Mix powder. Mix by swirling or inverting the bottle 10 times. Allow contents to dissolve for 10 minutes at room temperature.
Note: After reconstitution of the GGT Reagent Mix, the entire kit should be stored at -20°C between uses. The frozen liquid components should be thawed and warmed to room temperature immediately before each use.

IMPORTANT: The reconstituted GGT Reagent Mix can be left at room temperature for short periods (30 – 60 minutes) prior to use. Between uses, the reconstituted GGT Reagent Mix should be stored at -20°C (for up to 6 months). Discard the GGT Reagent Mix 6 months after reconstitution.

To obtain higher sensitivity measurements use a temperature controlled plate reader, if available. Adjust the plate reader temperature control to 37°C and equilibrate the GGT Reagent Mix to 37°C for 10 minutes before use.

Preparation of pNA Control Dilutions for Standard Curve (*Optional**)

Vortex mix the pNA Control tube for 20 seconds. Label six microfuge tubes: 1, 2, 3, 4, 5, Neg. Then make 6 **serial** dilutions of the pNA Control using the pNA Dilution Buffer as described in the table below.

NOTE: There is enough material to construct 2 Standard Curves. Make the pNA Control Dilutions for the Standard Curve fresh each time that the Standard Curve is performed. After each dilution, briefly mix the tube before performing the next dilution.

Standard Tube #	Preparation	GGT (IU/L)
1	Add 150 µL of pNA Control.	250
2	Add 100 µL from Standard Tube #1 + 100 µL of pNA Dilution Buffer. Mix thoroughly.	125
3	Add 100 µL from Standard Tube #2 + 150 µL of pNA Dilution Buffer. Mix thoroughly.	50
4	Add 100 µL from Standard Tube #3 + 100 µL of pNA Dilution Buffer. Mix thoroughly.	25
5	Add 100 µL from Standard Tube #4 + 150 µL of pNA Dilution Buffer. Mix thoroughly.	10
6 (Neg)	Add 100 µL of pNA Dilution Buffer.	0

**Only needed for the generation of the Standard Curve.*

Assay Protocol

1. Allow allow assay components to warm to room temperature before use.
2. Add 10 μL of each sample or standard (in duplicate) to the microplate wells.
3. Add 240 μL of GGT Reagent Mix to the wells. (✍ Using a multichannel pipet or repeating pipettor is recommended).
4. Immediately measure the absorbance of each sample at 405 nm. Exactly 10 minutes later, measure the absorbance again.
5. For each point, determine the increase in absorbance per 10 minute time interval by subtracting the absorbance at the initial time point from the absorbance at the 10 minute time point.

DATA ANALYSIS

Determination of γ-Glutamyl Transferase Activity in Serum Samples

Average the duplicate absorbance increases for each sample to obtain the average increase in absorbance for each.

Using the supplied materials and the procedure described above, the concentration of GGT (units per liter) can be determined by multiplying the average increase in absorbance in 10 minutes by 353.

For example, if an absorbance increase of 0.1 is observed over the 10 min interval, the GGT enzyme concentration in the sample would be 353 x 0.1 = 35.3 U/L.

Note: If the GGT level of a sample is greater than 400 U/L, dilute the sample 1:1 with PBS and repeat the measurement.

Standard Curve Construction *(Optional)*

A calibration curve to confirm assay linearity can be constructed using the pNA Control dilutions as described below:

1. For each calibration point, calculate the *average absorbance change.* To do this, subtract the average **10 minutes** absorbance value of the "**Neg**" (no pNA) point from the average **10 minutes** absorbance value of each point. This calculation should include subtracting the average 10 minutes absorbance of the "Neg" value from itself, which is approximately zero.
2. For each standard, plot the average absorbance change along the y-axis (from lowest in value to highest in value) and the GGT concentration on the x-axis.

SELECTED REFERENCES

Vázquez-Medina, J. P., et al. (April 2011) Prolonged fasting increases glutathione biosynthesis in post weaned northern elephant seals. J. Exp. Biol., 214: 1294 - 1299.

XpressBio
P.O. BOX 458
Thurmont, MD 21788
Tel: 301.228.2444
Fax: (301) 560.6570

Made in USA
info@xpressbio.com
www.xpressbio.com