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IDTox™ γ-Glutamyl Transferase (GGT) Enzyme Assay Kit

SUP6011

Enzyme Immunoassay for the determination of the γ-glutamyl transferase enzyme in serum samples.

PRODUCT DESCRIPTION

The IDTox™ 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 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 features of the kit are:

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

PROCEDURE OVERVIEW

This kit uses an enzymatic reaction to measure enzyme levels in serum. The assay measures the cleavage of a specific GGT substrate (γ –glutamyl-p-nitroanilide) by the enzyme. The production of the p-nitroanailine (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.

KIT REAGENTS SUPPLIED

The IDTox™ 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, when properly stored is noted on the label. 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 Reagent Mix"

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

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (405 nm).
Centrifuge to prepare serum samples.
Deionized or distilled water.
1.5 ml microfuge tubes.
Multichannel pipette or repeating pipettor (*recommended but not required*).
PBS

SENSITIVITY (Serum Detection Limit)

7 U/l

For *in vitro* research use. CAUTION: Not for human or animal therapeutic use.
Uses other than the labeled intended use may be a violation of local law.

WARNINGS AND PRECAUTIONS

It is strongly recommended 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 coming with the kit.

Do note use the kit past the expriation date. 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. Use only distilled or deionized water since water quality is very important. 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 also be avoided. Wear gloves when performing the procedure.

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ASSAY PROCEDURE

PREPARATION OF SAMPLES

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.

Set up

Allow all reagents and the microtitre plate to warm up to room temperature before use.

Preparation of Reagent Mix

Reconstitution: Add exactly 27 ml of deionized or distilled water to the Reagent Mix powder. Mix by swirling or inverting the bottle 10 times. Allow contents to dissolve for 10 minutes at room temperature. The reconstituted Reagent Mix can be left at room temperature for short periods (30-60 minutes) prior to use. Between uses the Reagent Mix should be stored at -20°C for up to 6 months. Allow the mix to warm up to room temperature for 30 – 60 minutes before use. Discard the mix 6 months after reconstitution. If a temperature controlled plate reader is available, adjust the plate reader temperature control to 37°C and equilibrate the Reagent Mix at 37°C for 10 minutes before use to obtain even higher sensitivity measurements.

Preparation of pNA Control Dilutions for Standard Curve – (optional*)

Vortex the pNA Control tube for 20 seconds. Label six microfuge tubes: 1, 2, 3, 4, 5,6 (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.

Sample Test Procedure

1. 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 Reagent Mix to the wells.
4. Measure the absorbance of each sample at 405 nm. Exactly 10 minutes later, measure 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.

CALCULATION OF RESULTS

Standard Curve Construction

NOTE: This optional Standard Curve provides a reference for the linear range of the assay. It is simply used as a test to show that the experiment was carried out correctly; e.g. proper dilutions, temperatures, times, etc. The Standard Curve IS NOT USED to determine the concentration of GGT in the samples; see **Determination of γ-Glutamyl Transferase Activity in Serum Samples** section below.

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 minute absorbance value of the "Neg" (no pNA) point from the average 10 minute absorbance value of each point. This calculation should include subtracting the average 10 minute absorbance of the "Neg" value from itself, which is approximately zero.

2. For each standard, plot the average corrected absorbance along the y-axis (from lowest in value to highest in value) and the GGT concentration on the x-axis.

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 decrease of 0.1 is observed over the 10 minute 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 then dilute the sample 1:1 with PBS and repeat the measurement.