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IDTox™ Triglyceride Enzyme Assay Kit

SUP6010

Enzyme Immunoassay for the determination of the Triglycerides in serum or plasma samples.

PRODUCT DESCRIPTION

Triglycerides are a prominent class of lipids found in blood. Triglycerides are used to form cell membranes and hormones, and play important roles in signal transduction pathways. The determination of serum triglycerides is an important marker for the diagnosis of lipemias. Elevated levels of triglycerides are strongly associated with vascular diseases such as stroke and atherosclerosis.

The IDTox™ Triglycerides Enzyme Assay Kit is a simple, direct and automation-compatible method for measuring triglyceride levels in serum samples. This kit uses a coupled enzymatic reaction scheme: triglyceride esters are first converted to glycerol and fatty acids. Next, glycerol is converted using ATP to glycerol-phosphate. Lastly, the glycerol-phosphate is enzymatically oxidized to for hydrogen peroxide, which is visibly detected (at 520 nm) by peroxidase using a chromogenic dye. The absorption measured at 520 nm, is proportional to the concentration of triglycerides in the sample. The kit also comes with a control solution containing a triglyceride standard (200 mg/dl) which can be used to calibrate the assay.

This kit provides direct determination of triglycerides in serum. In addition, the kit can be used to analyze the pharmacological effects of drugs on triglyceride metabolism.

The kit contains sufficient materials to rapidly test 42 serum or plasma samples in duplicate.

The kit uses a spectrophotometric assay to detect triglycerides directly from serum or plasma samples. The unique features of the kit are:

- High sensitivity and low detection limit (5 mg/dl).
- A rapid (10 minutes) and robust enzyme-based assay which does not require expensive instrumentation.
- High reproducibility.
- Requires only 5 µl of serum.

KIT REAGENTS SUPPLIED

The IDTox™ Triglycerides Assay Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). Store the kit (except for the microplate) at 4 °C. The shelf life is 6 months when the kit is properly stored. Once the Reagent Mix is reconstituted with water, the shelf-life is 2 months when properly stored.

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	RT
Reagent Mix	Vial	2-8ºC
Triglyceridee Standard (200 mg/dl)	800 µl	2-8ºC

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (520 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 (phosphate buffer saline, pH 7.3)
Methanol

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.

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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 that is supplied with the kit.

Add standards to plate only in the order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve. 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. Do not use the kit past the expiration date.

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ASSAY PROCEDURE

SAMPLE PREPARATION

Serum

1. Carefully collect whole blood in a 1.5 ml microfuge tube or serum collection tube making sure to avoid hemolysis as it may release erythrocyte triglycerides into the serum.
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. Triglycerides in serum are stable for several days at room temperature (4°C) and six months when frozen and properly protected against evaporation.

Note:

1. Samples with values above 600 mg/dl should be diluted 1:1 with PBS and re-tested. Multiply the results by two.
2. Grossly lipemic serums require a sample blank. Add 5 µl of sample to 250 µl saline, mix and read the absorbance against water. Subtract this reading value from the absorbance of each serum sample to obtain the corrected reading.

TRIGLYCERIDES DETECTION PROTOCOL

Set up

1. Allow reagents to come to room temperature.

Preparation of Reagent Mix

To reconstitute the Reagent Mix, add exactly 27ml 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.

NOTE: The reconstituted Reagent Mix can be left a room temperature for short periods (30-60 minutes) prior to use. Between uses, the reconstituted Reagent Mix should be stored at 4°C (for up to 2 months). Discard the Reagent Mix 2 months after reconstitution.

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Preparation of Standard Dilutions for Standard Curve

1. Label 6 microfuge tubes: 1, 2, 3, 4, 5, 6 (Neg).
2. Make 6 dilutions of the Triglyceride Standard using methanol as described in the table below. After dilution, briefly mix each tube before performing the next dilution.

NOTE: There is enough material to construct 3 Standard Curves. Make the Triglyceride Control Dilutions for the standard curve fresh each time that the standard curve is preformed.

Tube #	Preparation	Triglyceride (mg/dL)
1	Add 100 µl of Triglyceride Standard	200
2	Add 75 µl of Triglyceride Standard 1 + 25 µl methanol. Mix thoroughly.	150
3	Add 50 µl of Triglyceride Standard 1 + 50 µl methanol. Mix thoroughly.	100
4	Add 25 µl of Triglyceride Standard 1 + 75 µl methanol. Mix thoroughly.	50
5	Add 10 µl of Triglyceride Standard 1 + 90 µl methanol. Mix thoroughly.	20
6 (Neg)	Use 100 µl of methanol	0

Assay Protocol

1. Add 5 µl of each sample or standard (in duplicate) to the microplate wells.
2. Add 250 µl of Reagent Mix to the wells.
3. Incubate at 37 °C for 5 minutes or room temperature for 10 minutes.
4. Measure the absorbance of each sample at 520 nm.

CALCULATION OF RESULTS

There is a linear relationship between the concentration of triglycerides in the sample and absorbance at 520 nm. Therefore, a standard curve used to calculate the triglycerides concentration in sera samples can be constructed by plotting the mean corrected absorbance values for each of the diluted triglycerides standards as a function of triglycerides concentration (mg/dl). The straight line which best fits the data of Standard Curve can be used to calculate the triglyceride concentration in each sample.