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IDTox™ Cholesterol Enzymatic Assay Kit

SUP6006

Enzyme Immunoassay for the determination of the Cholesterol in serum samples.

PRODUCT DESCRIPTION

Cholesterol is a prominent sterol lipid present in cell membranes, bile, brain tissue, etc. and is transported in the bloodstream of all warm-blooded animals. It is used to form cell membranes and hormones, and plays important roles in cell signaling. Cholesterol is a chemical precursor to bile acids, steroids, and vitamin D. The determination of serum cholesterol is an important marker for the diagnosis of lipemias. Other conditions such as thyroid diseases influence cholesterol levels. Elevated levels of cholesterol (hypercholesterolemia) have been strongly associated with cardiovascular diseases such as atherosclerosis; low levels of cholesterol (hypocholesterolemia) may be linked to cancer, depression and cerebral hemorrhage.

The IDTox™ Cholesterol Enzymatic Assay Kit is a simple, direct and automation-compatible method for measuring cholesterol levels. This kit uses a coupled enzymatic reaction scheme: cholesterol esters are first converted to cholesterol and fatty acids. Next, cholesterol is oxidized with O_2 to form cholesten-3-one + H_2O_2. Lastly, the hydrogen peroxide is reacted with 4-aminoantipyrine and p-HBS to yield quinoneimine (red dye) and water. The absorption measured at 520 nm, is proportional to the concentration of cholesterol in the sample. The kit also comes with a control solution containing a cholesterol standard (200 mg/dl) which can be used to calibrate the assay.

This kit provides direct determination of cholesterol in serum, plasma, and other fluid samples. In addition, the kit can be used to analyze the pharmacological effects of drugs including siRNA and miRNA on cholesterol metabolism. For example, the impact of siRNA targeting ApoB1 can be monitored using this kit.

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

The kit uses a spectrophotometric assay to detect cholesterol directly from serum samples, enabling researchers to detect cholesterol levels in animal serum and other tissue matrices. The unique features of the kit are:

- High sensitivity and low detection limit (25 mg/dl).
- A rapid (10 minutes) and robust enzyme-based assay which does not require expensive instrumentation.
- High reproducibility.

PROCEDURE OVERVIEW

After preparing the sera, the assay is performed by adding Reagent Mix into microplate wells containing 5 µl sera. After a brief incubation, the absorbance of each well at 520 nm is then measured using a plate reader. The concentration of cholesterol in each sample is then directly determined from the 520 nm absorbance.

KIT REAGENTS SUPPLIED

The IDTox™ Cholesterol 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 of the kit is noted on the label when the kit is properly stored.

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	RT
Reagent Mix	bottle	2-8ºC
Cholesterol Standard (200 mg/dl)	300 µl	2-8ºC

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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

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. 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. Anticoagulants such as fluoride and oxalate will result in false low values. The assay is not influenced by hemoglobin values up to 200mg/dl or by bilirubin levels up to 10mg/dl. Interference from grossly hemolyzed specimens is correctable by use of a serum/plasma blank.

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ASSAY PROCEDURE

SAMPLE PREPARATION

1. Carefully prepare at least 20 µl of sera using standard production procedure (if determinations are performed in singlet then 10 µl is sufficient). Avoid hemolysis as it may release erythrocyte cholesterol into the serum.
2. Cholesterol in serum is reported stable for seven days at room temperature (18-30 ^{o}C) and six months when frozen and properly protected against evaporation.

Note:
1. Samples with values above 200 mg / dl should be diluted 1:1 with PBS and re-tested. Multiply 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.

CHOLESTEROL DETECTION TESTS PROTOCOL

Set up

1. Allow reagents to come to room temperature. Turn on the plate reader and allow lamp to warm up. Adjust the wavelength of the plate reader to 520 nm.

Reagent Preparation

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. The Reagent Mix is stable for 3 months after reconstitution with water.

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Note:
The reconstituted Reagent Mix can be left ar room temperature for short periods (30-60 minutes) prior to use. Between uses the reconstituted Reagent Mix should be stored at 4oC (for up to 3 months). Discard the Reagent Mix 3 months after reconstitution.

Preparation of Standard Dilutions for Standard Curve

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

Tube #	Calibration Standard (200 mg/dl)	Methanol	Total Volume
1	100 µl	0 µl	100 µl
2	80 µl	20 µl	100 µl
3	60 µl	40 µl	100 µl
4	30 µl	70 µl	100 µl
5	10 µl	90 µl	100 µl
6 (Neg)	0 µl	100 µl	100 µl

3. 5 µl of each diluted standard is used per reaction (see below).

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 10 minutes.
4. Measure the absorbance of each sample at 520 nm.

CALCULATION OF RESULTS

There is a linear relationship between the concentration of cholesterol in the sample and absorbance at 520 nm. Therefore, a standard curve used to calculate the cholesterol concentration in sera samples can be constructed by plotting the mean corrected absorbance values for each of the diluted cholesterol standards as a function of cholesterol concentration. The standard curve provides a reference for the linear range of the assay.