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IDTox™ LDL Cholesterol Enzymatic Assay Kit

SUP6013

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

PRODUCT DESCRIPTION

LDL (low-density lipoprotein) is one type of lipid/protein particle used to transport lipids such as triglycerides and phospholipids throughout the bloodstream. LDL particles also contain significant amounts of cholesterol. Increased levels of LDL cholesterol are associated with atherosclerosis and coronary artery disease. For this reason, measuring LDL cholesterol levels has been shown to often predict the occurrence of heart disease.

The IDTox™ LDL Cholesterol Assay Kit is a simple microplate-based method for measuring LDL cholesterol levels from serum or plasma. This kit uses a specific detergent formulation to selectively dissolve non-LDL lipoprotein particles (HDL, VLDL and chylomicrons) while leaving LDL particles intact. The dissolved cholesterol is degraded by the cholesterol esterase and cholesterol oxidase enzymes. Next a second detergent is added to the sample to solubilize the remaining LDL particles. The soluble cholesterol and cholesterol esters are oxidized by cholesterol oxidase to produce hydrogen peroxide. The hydrogen peroxide product then reacts with bis(4-sulfobutyl)-m-toluidine and 4-aminoantipyrine to form a colored product. The resulting color change is measured at 550 nm and is proportional to the amount of LDL cholesterol originally present in the sample.

The kit also comes with a control solution containing a LDL cholesterol standard which can be used to calibrate the assay and generate a standard curve. The kit contains sufficient materials to rapidly test 42 serum samples in duplicate.

The IDTox™ LDL Cholesterol Assay Kit is a plate-based colorimetric assay for the determination of LDL cholesterol in serum or plasma samples.

The kit uses a spectrophotometric assay to detect LDL directly from serum samples, enabling researchers to measure LDL cholesterol levels in rodent serum. The unique features of the kit are:

- High sensitivity and low detection limit (10 mg/dl)
- A rapid 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 LDL Cholesterol Reagent A into microplate wells containing 2 µl sera to dissolve and degrade non-LDL cholesterol. After a brief incubation, a second regent is added to the reaction to selectively generate a colored reaction product (550 nm) from the LDL cholesterol in the sample. The absorbance of each well at 550 nm is then measured using a plate reader. The concentration of LDL cholesterol in each sample is then directly determined from the 550 nm absorbance.

KIT REAGENTS SUPPLIED

The IDTox™ LDL 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)	2-8ºC
LDL Cholesterol Reagent A	2 x 10 ml	2-8ºC
LDL Cholesterol Reagent B	7 ml	2-8ºC
Cholesterol Standard (200 mg/dl)	800 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. Those containing citrate should not be used. The assay is not influenced by hemoglobin values up to 200 mg/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 10 µl of serum (or plasma) using standard production procedure (if determinations are performed in singlet then 5 µl is sufficient).
2. LDL cholesterol in serum is reported stable for five days at 4°C and six months when frozen.

Note:

Samples with LDL cholesterol levels above 500 mg/dl should be diluted with PBS or normal saline and re-tested. (Multiply results by the dilution factor).

LDL CHOLESTEROL DETECTION TESTS PROTOCOL

Set up

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.

Preparation of Standard Dilutions for Standard Curve

1. Label 6 microfuge tubes: 1, 2, 3, 4, 5 and 6.
2. Serially dilute the Cholesterol Standard using methanol as described in the table below. After dilution, briefly mix each tube.

Tube	Vol of Standard (µl)	Vol Methanol (µl)	Total Vol (µl)	Equiv LDL Chol Conc (mg/dl)

1	0	100	100	0
2	20	80	100	50
3	40	60	100	100
4	60	40	100	150
5	80	20	100	200
6	100	0	100	250

Important notes:
Due to analyte solubility issues, the volume used for the standard curve is larger than the sample volume used for serum samples: Use 15 µl of diluted standards in the reaction and only 2 µl serum in each reaction.
For serum samples, mix 2 µl serum with LDL Cholesterol Reagent A first. For standards, mix 15 µl of standard with LDL Cholesterol Reagent B first.

Assay Protocol
1. Add 200 µl of LDL Cholesterol Reagent A to the wells.
2. Carefully add 2 µl of each sample (in duplicate) to the microplate wells.
3. Incubate at 37°C for 5 minutes.
4. Add 67 µl of LDL Cholesterol Reagent B to the wells. Mix gently.
5. Incubate at 37°C for 5 minutes.
6. Measure the absorbance of each sample at 550 nm.

CALCULATION OF RESULTS
Procedure to Construct a Standard Curve
Important note: Add the reagents in reverse order (first LDL Cholesterol Reagent B, then LDL Cholesterol Reagent A) for the standards.

1. Add 15 µl of each standard (Tubes #1 - 6, in duplicate) to the microplate wells.
2. Add 67 µl of LDL Cholesterol Reagent B to the wells.
3. Incubate at 37°C for 5 minutes.
4. Add 200 µl of LDL Cholesterol Reagent A to the wells.
5. Incubate at 37°C for 5 minutes.
6. Measure the absorbance of each sample at 550 nm.

LDL Cholesterol Concentration Calculation
There is a linear relationship between the concentration of LDL cholesterol in the sample and absorbance at 550 nm. Therefore, a standard curve used to calculate the LDL cholesterol concentration in sera samples can be constructed by plotting the mean corrected absorbance values for each of the diluted LDL cholesterol standards as a function of LDL cholesterol concentration.