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IDTox™ HDL Cholesterol Enzymatic Assay Kit

SUP6014

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

PRODUCT DESCRIPTION

High-density lipoprotein (HDL) is one of the five major groups of lipoproteins which enables lipids like cholesterol and triglycerides to be transported within the water-based bloodstream. HDL particles are primarily involved in the uptake and transport of cholesterol from peripheral tissue to the liver for excretion or re-utilization. This reverse cholesterol transport is a proposed cardio protective mechanism, as low levels of HDL cholesterol have been linked to increased risk of coronary heart disease and coronary artery disease. For this reason, measuring HDL cholesterol levels has been shown to often identify those at a greater risk for heart disease.

This kit uses a specific reagent formulation to selectively stabilize non-HDL lipoprotein particles (LDL, VLDL and chylomicrons) while leaving HDL particles untouched. Next a second reagent containing a detergent and modified enzymes selectively reacts with the cholesterol present only in the HDL particles to form hydrogen peroxide. The hydrogen peroxide product then reacts with N-(2-hydroxy-3-sulfopropyl)-3,5-dimethyoxyaniline to form a colored product. The resulting color change is measured at 610 nm and is proportional to the amount of HDL cholesterol originally present in the sample.

The kit also comes with a HDL 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 kit uses a spectrophotometric assay to detect HDL directly from serum samples, enabling researchers to measure HDL 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 HDL Cholesterol Reagent A into microplate wells containing 3 µl sera to stabilize non-HDL particles. After a brief incubation, a second regent is added to the reaction to selectively generate a colored reaction product from the HDL cholesterol in the sample. The absorbance of each well at 610 nm is then measured using a plate reader. The concentration of HDL cholesterol in each sample is then directly determined from the 610 nm absorbance.

KIT REAGENTS SUPPLIED

The IDTox™ HDL 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
HDL Cholesterol Reagent A	24 ml	2-8ºC
HDL Cholesterol Reagent B	8 ml	2-8ºC
Cholesterol Standard (200 mg/dl)	800 µl	2-8ºC

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (520 nm).
Centrifuge to prepare serum samples.

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.

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. HDL cholesterol in serum is reported stable for five days at 4°C and six months when frozen.

Note:

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

HDL CHOLESTEROL DETECTION 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 Ethanol (µl)	Total Vol (µl)	Equiv HDL Chol Conc (mg/dl)
1	0	100	100	0
2	6.25	93.75	100	10
3	12.5	87.5	100	20
4	25	75	100	40
5	50	50	100	80
6	100	0	100	160

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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 3 µl serum in each reaction

Assay Protocol

1. Add 225 µl of HDL Cholesterol Reagent A to the wells.
2. Carefully add 3 µl of each sample (in duplicate) or 15 µl each standard (in duplicate) to the microplate wells.
3. Incubate at 37°C for 5 minutes.
4. Add 75 µl of HDL Cholesterol Reagent B to the wells. Mix gently.
5. Incubate at 37°C for 5 minutes.
6. Measure the absorbance of each sample at 610 nm.

HDL Cholesterol Concentration Calculation
There is a linear relationship between the concentration of HDL cholesterol in the sample and absorbance at 610 nm. Therefore, a standard curve used to calculate the HDL cholesterol concentration in sera samples can be constructed by plotting the mean corrected absorbance values for each of the diluted HDL cholesterol standards as a function of HDL cholesterol concentration