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Datasheet for ABIN2345055

HDL and LDL/VLDL Cholesterol Assay Kit

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Overview

Quantity:	192 tests
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	HDL and LDL/VLDL Cholesterol Assay Kit measures the HDL and LDL/VLDL cholesterol levels within serum or plasma samples.
Sample Type:	Serum, Plasma
Detection Method:	Fluorometric
Characteristics:	HDL and LDL/VLDL Cholesterol Assay Kit is a simple fluorometric assay that can measure the amounts of HDL and LDL/VLDL cholesterol present in plasma or serum samples within a 96-well microtiter plate format. The assay will detect total cholesterol (cholesteryl esters plus free cholesterol) in the presence of cholesterol esterase or only free cholesterol in the absence of the esterase enzyme. Each kit provides sufficient reagents to perform up to 192 assays, including blanks, cholesterol standards and unknown samples. Sample cholesterol concentrations are determined by comparison with a known cholesterol standard. Cholesteryl esters can be quantified by subtracting the free cholesterol values from the total cholesterol value.
Components:	1. 96-well Microtiter Plate : Two 96-well clear bottom black plates. 2. Cholesterol Standard : One 50 µL tube of a 10 mM cholesterol solution in ethanol. 3. Assay Diluent (5X) : One 100 mL bottle. 4. Fluorescence Probe : One 200 µL tube in DMSO. 5. HRP : Two 100 µL tubes of 100 U/mL solution each in glycerol. 6. LDL Precipitation Solution (2X) : Two 20 mL bottles. Box 2 (shipped on blue ice packs)

Product Details

Material not included:	1. Distilled or deionized water 2. 1X PBS 3. Microcentrifuge 4. Microcentrifuge tubes 5. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips 6. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips 7. Multichannel micropipette reservoir 8. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range. 9. Superoxide dismutase (optional) 5

Target Details

Background:	Cholesterol is a lipid sterol that is produced in and transported throughout the bloodstream in eukaryotes. Cholesterol is a critical compound used in the structure of cell membranes, hormones, and cell signaling. It is an essential component of animal cell structure in order to maintain permeability and fluidity. Cholesterol is a precursor for steroid hormones, including the adrenal gland hormones cortisol and aldosterone, sex hormones progesterone, estrogens, and testosterone, as well as bile acids, and vitamin D. Cholesterol is transported around the body within lipoproteins, which are submicroscopic particles composed of lipid and protein held together by noncovalent forces. Their general structure is that of a putative spheroidal microemulsion formed from an outer layer of phospholipids, unesterified cholesterol, and proteins, with a core of neutral lipids, predominately cholesteryl esters and triacylglycerols (TAG). Lipoprotein's main function is to transport these lipids around the body in the blood. Lipoprotein particles have hydrophilic groups of phospholipids, cholesterol, and apoproteins directed outward. Such characteristics make them soluble in the salt water-based blood pool. Triglyceride-fats and cholesterol esters are carried internally, shielded from the water by the phospholipid monolayer and the apoproteins. The interaction of the proteins forming the surface of the particles with enzymes in the blood, with each other, and with specific proteins on the surfaces of cells determine whether triglycerides and cholesterol will be added to or removed from the lipoprotein transport particles. Lipoproteins have cell-specific signals that direct the lipids they transport to certain tissues. For this reason, lipoproteins exist in different forms within the blood based on their density. These include chylomicrons, very-low density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), low- density lipoproteins (LDLs), and high-density lipoproteins (HDLs). The higher the lipid content in a lipoprotein, the less dense it is. Cholesterol exists within a lipoprotein as a free alcohol and as a fatty cholesteryl ester, which is the predominant form of cholesterol transport and storage. High blood levels of LDLs are associated with health problems and cardiovascular disease. For this reason, LDL is often

referred to as the "bad cholesterol." LDL particles that accumulate within arteries can form plaques over time, which can increase chances of a stroke, heart attack, or vascular disease. HDL particles are able to remove cholesterol from within arteries and transport it back to the liver for re- utilization or excretion, which is the main reason why the cholesterol carried within HDL particles is sometimes called "good cholesterol." Monitoring circulatory levels of different lipoproteins is critical to the diagnosis of lipid transport disorders such as atherosclerosis.

Application Details

Application Notes: Optimal working dilution should be determined by the investigator.

Comment:

- Allows you to separate and independently test HDL and LDL/VLDL fractions
- Suitable for use with serum or plasma
- Cholesterol standard included

Protocol: The kit provides reagents for separating and quantifying HDL and LDL/VLDL cholesterol. The assay is based on the enzyme driven reaction that quantifies both cholesterol esters and free cholesterol. Cholesterol esters are hydrolyzed via cholesterol esterase into cholesterol, which is then oxidized by cholesterol oxidase into the ketone cholest-4-en-3- one and hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific fluorescence probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of cholesterol standard within the 96-well microtiter plate format. Samples and standards are incubated for 45 minutes and then read with a standard 96-well fluorometric plate reader .

Reagent Preparation:

- 1X Assay Diluent: Warm the Assay Diluent (5X) to room temperature prior to using. Dilute the Assay Diluent (5X) with deionized water by diluting the 100 mL Diluent with 400 mL deionized water for 500 mL total. Mix to homogeneity. Store the 1X Assay Diluent at 4 °C up to six months.
- Cholesterol Esterase: Reconstitute the powder with 200 µL of 1X Assay Diluent. Vortex vigorously until dissolved. Prepare aliquots and store at -20 °C to avoid multiple freeze thaws of the reconstituted powder.
- Cholesterol Reaction Reagent: Prepare the reagent by diluting the Cholesterol Oxidase 1:50, HRP 1:50, Fluorescence Probe 1:50, and Cholesterol Esterase 1:250 in 1X Assay Diluent. (eg. For 100 assays, combine 100 µL of Cholesterol Oxidase, 100 µL of HRP, 100 µL Fluorescence Probe, and 20 µL Cholesterol Esterase with 1X Assay Diluent to 5 mL total solution). Mix thoroughly and protect the solution from light. For best results, place the Cholesterol Reaction Reagent on ice and use within 30 minutes of preparation. Do not store the Cholesterol Reaction Reagent solution. Notes: 1. If testing for the concentration of free cholesterol only, omit the addition of Cholesterol Esterase from the Cholesterol Reaction Reagent solution. 2. The Fluorescence Probe is light sensitive and must be stored

	accordingly.

Sample Preparation:

Samples should be used immediately or stored at -80 °C prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples. • Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the serum layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80 °C. Perform dilutions in 1X Assay Diluent. Cholesterol levels in serum average about 3 % higher in value than in the corresponding plasma pair (Ref. 2). • Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4 °C for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80 °C. Perform dilutions in 1X Assay Diluent. Notes:

1. Samples with NADH concentrations above 10 μM and glutathione concentrations above 50 μM will oxidize the probe and could result in erroneous readings. To minimize this interference, it 6 is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL.
2. Avoid samples containing DTT or β-mercaptoethanol since the fluorescence probe is not stable in the presence of thiols (above 10 μM). Preparation of HDL and LDL/VLDL Fractions
   1. Add 200 μL of sample (serum or plasma) to a microcentrifuge tube. Add 200 μL of the Precipitation Reagent and mix well by vortexing. Allow mixture to incubate 5-10 minutes at room temperature (precipitation will occur).
   2. Centrifuge the mixture at 2000 x g (~5000 rpm) for 20 minutes (pellet should be visible). Slowly and carefully transfer the supernatant (HDL fraction) into a new tube, leaving the pellet (LDL/VLDL fraction). Resuspend and dissolve the pellet in 400 μL of PBS, vortexing well. Ensure that the pellet (LDL/VLDL fraction) is completely dissolved before testing.
   3. Further dilute the HDL or LDL/VLDL fraction samples 1:25 (1:50 final dilution) to 1:100 (1:200 final dilution) in 1X Assay Diluent before running the assay. Assay immediately and do not store solutions. Notes:
      1. Samples with NADH concentrations above 10 μM and glutathione concentrations above 300 μM will oxidize the probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL.
      2. Avoid samples containing DTT or β-mercaptoethanol since the fluorescence probe is not stable in the presence of thiols (above 10 μM).

Assay Procedure:

Each cholesterol standard and sample should be assayed in duplicate or triplicate. A freshly

	prepared standard curve should be used each time the assay is performed. 1. Add 50 µL of the diluted cholesterol standards or samples to the 96-well microtiter plate. 2. Add 50 µL of the prepared Cholesterol Reaction Reagent to each well and mix the well contents thoroughly. 3. Cover the plate wells to protect the reaction from light. Incubate the plate for 45 minutes at 37 °C. 4. IMMEDIATELY read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range. 5. Calculate the concentration of cholesterol within samples by comparing the sample RFU to the cholesterol standard curve.
Calculation of Results:	1. Calculate the average fluorescence values for every standard, control, and sample. Subtract the average zero standard value from itself and all standard and sample values. This is the corrected fluorescence. 2. Plot the corrected fluorescence for the standards against the final concentration of the cholesterol standards from Table 1 to determine the best curve. See Figure 3 for an example standard curve. 3. Determine the cholesterol concentration of the samples with the equation obtained from the linear regression analysis of the standard curve. Substitute the corrected fluorescence values for each sample. Remember to account for all dilution factors. Sample corrected fluorescence Total, HDL, LDL/VLDL (µM) = x Sample dilution Cholesterol Slope Cholesteryl Ester (µM) = Total Cholesterol - Free Cholesterol Total Cholesterol (Unfractionated) (µM) ~ HDL Cholesterol (µM) + LDL/VLDL Cholesterol (µM) Note: For the conversion of results from µM to mg/dl, divide the cholesterol concentration (µM) by 25.9.
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C
Storage Comment:	Upon receipt, store the Cholesterol Standard, Fluorescence Probe, HRP, Cholesterol Oxidase, and Cholesterol Esterase at -20°C. The Fluorescence Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles. Store the remaining kit components at 4°C.

Publications

Product cited in:	Jang, Bae, Kim, Cho, Yuk, Han, Youn, Kwon, Hwang, Kim et al.: "The herbal formula KH-204 is protective against erectile dysfunction by minimizing oxidative stress and improving lipid profiles in a rat model of erectile dysfunction induced by ..." in: **BMC complementary and**

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Angelovich, Shi, Zhou, Maisa, Hearps, Jaworowski: "Ex vivo foam cell formation is enhanced in monocytes from older individuals by both extrinsic and intrinsic mechanisms." in: **Experimental gerontology**, Vol. 80, pp. 17-26, (2016) (PubMed).

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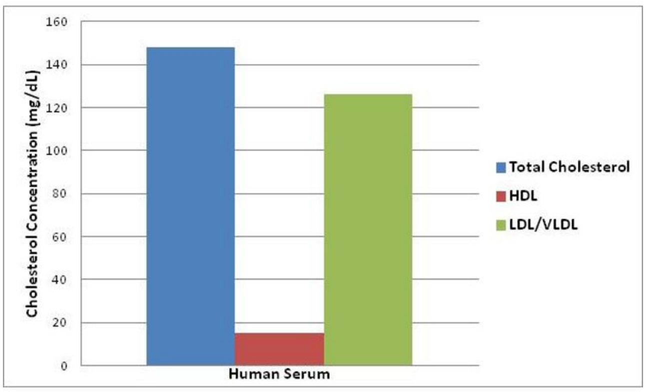
Maisa, Hearps, Angelovich, Pereira, Zhou, Shi, Palmer, Muller, Crowe, Jaworowski: "Monocytes from HIV-infected individuals show impaired cholesterol efflux and increased foam cell formation after transendothelial migration." in: **AIDS**, Vol. 29, Issue 12, pp. 1445-57, (2015) (PubMed).

There are more publications referencing this product on: Product page

Images



ELISA

Image 1. holesterol Values of Human Serum Tested Using the HDL and LDL/VLDL Cholesterol Assay Kit.

