Catalog #79980

V.3

Mouse LDL-Cholesterol Kit Instructions

For the quantitative determination of LDL-cholesterol
in mouse serum or plasma

Catalog #79980
96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Mouse LDL-Cholesterol kit is for the quantitative determination of LDL-cholesterol in mouse serum or plasma. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

It has been shown that most of cholesterol stored in atherosclerotic plaques originates from LDL. For this reason the LDL-cholesterol concentration is considered to be the most important predictors, of all single parameters, with respect to coronary atherosclerosis.

Accurate measurement of LDL-cholesterol is of vital importance in therapies which focus on lipid reduction to prevent atherosclerosis or reduce its progress and to avoid plaque rupture.

C. Principle of the Assay

Crystal Chem's Mouse LDL-cholesterol assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. LDL, VLDL, and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER), whereas HDL reacts with the enzymes. Addition of the second reagent containing a specific detergent releases LDL from the PVS/PEGME complex. The released LDL reacts with the enzymes to produce H_2O_2 which is quantified by the Trinder reaction.

D. Kit Storage

1. Upon receipt of the Mouse LDL-Cholesterol Kit, store it at 2-8°C (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
CC1	Reagent CC1 (liquid)	1 X 25 mL
CC2	Reagent CC2 (liquid)	1 X 8 mL
CAL1	Calibrator 1 (lyophilized)	1 X 1 mL

E.2. Materials required but not provided

Microplates
Micropipettes and disposable tips
Clean glass tubes and test tube racks
Volumetric flasks
Incubator (37ºC)
Distilled water
Microplate reader or spectrophotometer (should read A_{600} values)
0.9% Saline

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Reagents are light-sensitive. Store in a dark place. Do not let bottles remain open. Keep containers tightly closed.
4. Do not use the reagents after the expiration date.

G. Maximizing Kit Performance

1. Given the small sample volumes required (3 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Use fresh mouse serum or plasma samples (EDTA, Citrate). Fasting and non-fasting samples can be used. Anticoagulants using heparin plasma should not be used.

I. Assay Procedure

I.1. Preparation of reagents

All reagents are provided ready-to-use and should be brought to room temperature for at least 30 minutes prior to use. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling

I.2. Preparation of samples, calibrators, and controls

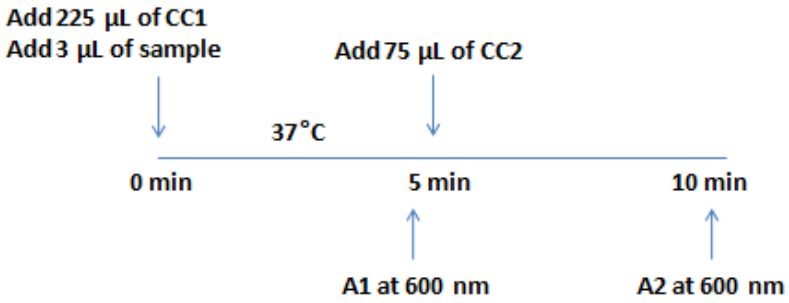
1. Reconstitute the calibrators as directed on the label in 1 mL of distilled water. To ensure complete reconstitution, equilibrate vials at room temperature for 30 minutes before first use.
   Note: Reconstituted calibrators are stable for 7 days when capped tightly and stored at 2-8°C. In addition to running the calibrator provided, the assay requires running a blank calibrator. 0.9% saline should be used for running the blank calibrator. Optional controls are sold separately (Cat# 79983). Controls should be reconstituted per the directions provided on the label.
2. Bring all samples, calibrators, and controls to room temperature.

I.3. Assay procedure

The procedure below reflects a manual procedure performed using a microplate reader. The procedure can be adopted to be run in a glass tube using a spectrophotometer. The assay can also be adopted to work on various automated analyzers. Please contact Crystal Chem for more information.

1. Add 225 µL of Reagent CC1 and 3 µL of sample, calibrator, or control into each well (as needed) of a microplate and mix well by repeated pipetting.
2. Place microplate in incubator (37ºC) and allow microplate to equilibrate to 37ºC over 5 minutes.
3. Measure absorbance using a plate reader (measure A_{600} values).
   ***Note**: The Mouse LDL-Cholesterol assay is an end-point assay and the first reading point A1 is right before the addition of reagent CC2.*
4. Pipette 75 µL of Reagent CC2 and mix well by repeated pipetting.
5. Measure the increase in absorbance after 5 minutes at 37°C using a plate reader (measure A_{600} values).

Figure 1. Summary of assay procedure



I.4. Determining the mouse LDL-cholesterol concentration

1. Calculate the change in absorbance ΔA (5 mins ~ 0 mins)

   $\Delta A = (OD_{600nm,\ 5\ mins}) - (OD_{600nm,\ 0\ mins})$

2. Using linear graph paper, construct the LDL-cholesterol calibration curve by plotting the mean change in absorbance value for the calibrator (incl. blank) on the Y axis versus the corresponding LDL-cholesterol concentration on the X axis.
   ***Note:** Calibrator value varies per lot and should be obtained from the calibrator label.*
3. Mouse LDL-cholesterol concentrations in the samples are interpolated using the calibration curve and mean change in absorbance values for each sample. This interpolation can be simplified using Equation 1 below. The LDL-cholesterol concentration is expressed as mg/dL. This unit of measure can be converted in mmol/L by multiplying the obtained concentration in mg/dL by 0.02586.
   ***Note:** Samples with a high mouse LDL-cholesterol concentration (245.0 mg/dL or higher) should be diluted with 0.9% saline and rerun.*

Equation 1. Calculation of LDL-cholesterol concentration

LDL-cholesterol concentration =

[(sample ΔA600 –blank ΔA600) / (cal Δ A600 – blank ΔA600)] × cal conc.

J. Performance characteristics

J.1. Assay range

The Mouse LDL-cholesterol assay has a linear range from 4.5 - 245.0 mg/dL.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

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