Low-density Lipoprotein/ Very-low-density Lipoprotein Microplate Assay Kit

User Manual

Catalog # CAK1115

(Version 1.3E)

Detection and Quantification of Low-density Lipoprotein/Very-low-density Lipoprotein (LDL/VLDL) Content in Serum, Plasma and other biological samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Lipoproteins transport the majority of plasma lipids including cholesterol and triglycerides. High-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) are the lipoproteins responsible for the vast majority of cholesterol transport in the blood. High LDL levels and low HDL levels are strongly associated with increased risk of adverse cardiovascular events.

In this kit, serum HDL and LDL/VLDL are first separated and then the cholesterol concentration of each is determined by a coupled enzyme assay. The products can be measured at a colorimetric readout at 550 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Precipitation Buffer	10 ml x 1	4 °C
Diluent	30 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard (4 mmol/L)	1 ml x 1	4 °C
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Note:

Enzyme: add 10 ml Diluent to dissolve before use.

Dye Reagent: add 10 ml Diluent to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer

IV. SAMPLE PREPARATION

1. For serum, plasma and other biological samples

Add 100 µl serum and 100 µl Precipitation Buffer into the microcentrifuge tube, mix, centrifuged at 3,000g 25 °C for 5 minutes. Remove all the supernatant from the microcentrifuge tube. Transfer 100 µl Assay Buffer to the microcentrifuge tube and mix by repeated pipetting.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Assay Buffer	--	--	10 µl
Enzyme	100 µl	--	--
Diluent	--	100 µl	100 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, 37 °C wait for 10 minutes, measured at 550 nm and record the absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the serum sample

$$LDL/VLDL\ (mmol/L) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

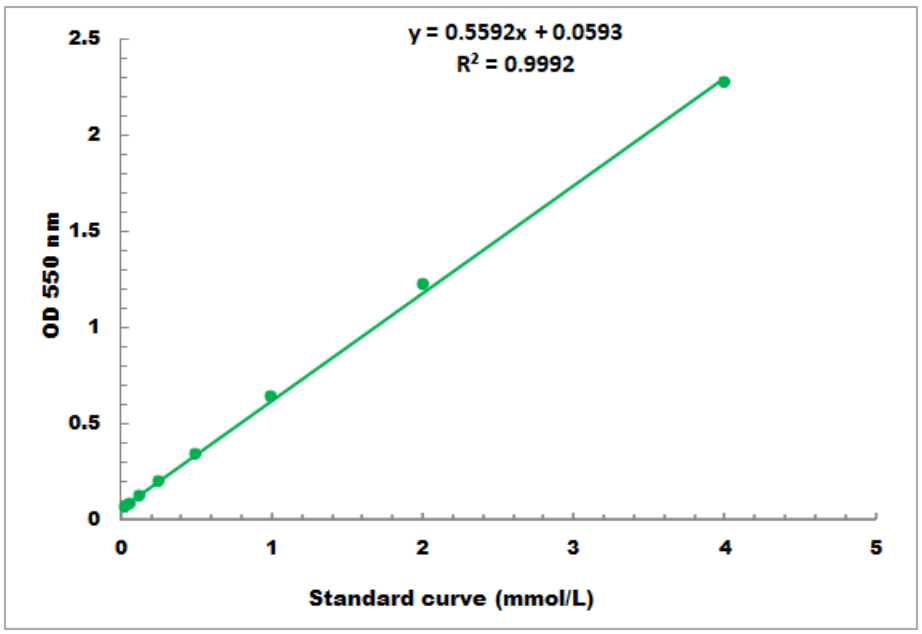
$C_{Standard}$: the concentration of Standard, 4 mmol/L;

$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.04 mmol/L - 4 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES