Low-density Lipoprotein/ Very-low-density Lipoprotein Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1115

(Version 1.4F)

Detection and Quantification ofLow-density Lipoprotein/Very-low-density Lipoprotein (LDL/VLDL)ContentinSerum, 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.
Low-density Lipoprotein/Very-low-density Lipoprotein Colorimetric Microplate Assay Kitis a sensitive assay for determining high-density Lipoproteinconcentration in various samples. In this assay, serum HDL and LDL/VLDL are first separated and then the cholesterol concentration of each is determined by a coupled enzyme assay.The productscan be measured at a colorimetric readout at 550 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Precipitation Buffer	10 mlx 1	4 °C
Diluent	30 mlx 1	4 °C
Enzyme	Powderx 1	-20 °C
Dye Reagent	Powderx 1	4 °C, keep in dark
Standard(9.8 mol/L)	0.2 ml x 1	4 °C, keep in dark
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Transfer 20 µl Standard (9.8M)into 960µl distilled water and mix well to generate 200 mmol/L as the stock standardsolution, which could be stored at 4°C for 2weeks. Then add 0.02 ml stock solution into 0.98 ml distilled water to generate 4 mmol/L as the top standardsolution. Then perform 2-fold serial dilutions of the top solution using distilled waterto make the standard curve. The concentration of standard curve could be 4/2/1/0.5/0.25/0.125/0.0625 mmol/L.

Enzyme: Add 10 ml Diluent to dissolve before use.Store at -20 °C for 1 month.

Dye Reagent: Add 10 ml Diluent to dissolve before use.Keep in dark and store at 4 °C for 2-3 weeks or -20 °C for 2 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For serum, plasma and other biological samples

Add 100 µlserum and100 µlPrecipitation Bufferinto the microcentrifuge tube, mix, centrifuged at 3,000g 25 °C for 5 minutes.Remove all the supernatant from the microcentrifuge tube. Transfer100 µlAssay Buffer to the microcentrifuge tube and mix by repeated pipetting.

VI. ASSAY PROCEDURE

Add following reagents intothe 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 recordthe absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

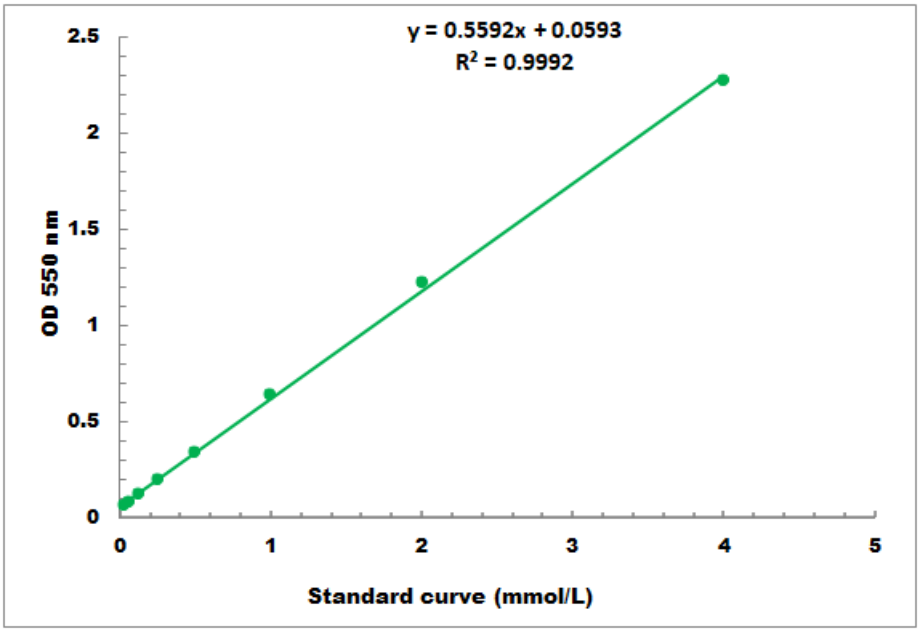
$C_{Standard}$: the standard concentration, mmol/L

$V_{Standard}$: the volume of standard in assay procedure, µl

V_{Sample}: the volume of sample in assay procedure, µl

VIII. 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