High-density Lipoprotein Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6721

Detection and Quantification of High-density Lipoprotein (HDL)Content inSerum, Plasma and other biological samples .

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

1. 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.

High-density Lipoprotein Colorimetric Microplate Assay Kit is a sensitive assay for determining high-density Lipoprotein concentration 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 products can be measured at a colorimetric readout at 550 nm.

2. 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
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3. 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
7. Hot air circulation oven

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Transfer 20 μL Standard (9.8M) into 960 μL distilled water and mix well to generate 200 mmol/L as the stock standardsolution , which could be stored at 4°C for2weeks. T hen 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 water to 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 1 0 mL Diluent to dissolve before use.Store at -20°C for 1 month.

Dye Reagent: Add 1 0 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.

5. Sample Preparation

1. For serum, plasma and other biological samples Add 100 μL serum and 100 μL Precipitation Buffer into the micro centrifuge tube, mix, centrifuged at 3,000g 25°C for 5 minutes.Take the supernatant into a new centrifuge tube for detection.

6. Assay Procedure

Add following reagents in to 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 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.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

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

Calculate the initial concentration according to sample preparation procedure .

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

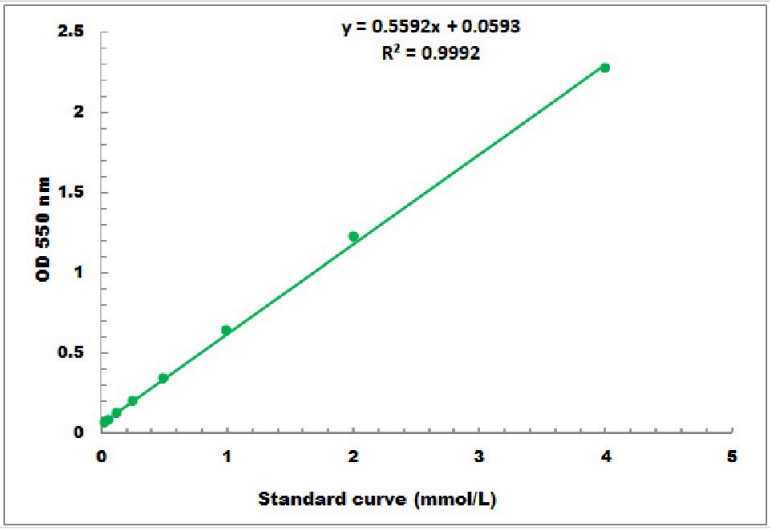
According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \times 2 \ (mmol/L)$$

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Standard}$	the standard concentration, mmol/L = μmol/ml
$V_{Standard}$	the volume of standard in assay procedure, μl
V_{Sample}	the volume of sample in assay procedure, μl

8. 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