MA-0124

HDL / LDL Cholesterol Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 538/587 nm, Store at -20°C)

Introduction:

HDL and LDL play a role in a variety of diseases. Low levels of HDL combined with high levels of LDL are associated with increased risk of cardiovascular events (plaque rupture, stroke, hypertension). Due to the high cost of directly measuring HDL and LDL/VLDL, a proxy, measuring cholesterol associated with these lipoproteins is commonly performed. By selectively precipitating LDL and measuring the cholesterol associated with the soluble and precipitated fractions a good estimate of the levels of each can be made. AkrivisBio's HDL/LDL Assay provides a simple sensitive method of quantifying HDL and LDL/VLDL cholesterol.

Assay Principle:

1- Serum samples are subjected to selective precipitation conditions separating HDL from LDL/VLDL fractions
2- Separated fractions are optionally treated with cholesterol esterase (for total cholesterol determination) or not (for free cholesterol only)
3- Cholesterol is oxidized with cholesterol oxidase forming cholestenone and hydrogen peroxide.
4- Hydrogen peroxide is used by peroxidase to oxidize ADHP to resorufin with a large increase in color (570 nm) and fluorescence (535/587 nm)

Assay Components:

Assay Buffer	25 ml	WM	MA-0124A
Precipitation Buffer	10 ml	NM	MA-0124B
ADHP Solution	200 µl	Red	MA-0124C
Cholesterol Oxidase/Peroxidase	lyoph	Green	MA-0124D
Cholesterol Esterase	lyoph	Blue	MA-0124E
Cholesterol Standard	100 µl	Yellow	MA-0124F

Storage and Handling: Store kit at -20°C. Centrifuge small vials for a few seconds prior to opening to pull vial contents to the bottom.
Assay Buffer and Precipitation Buffer: Warm to room temperature before use. Store at 4°C
ADHP Solution: Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
Cholesterol Esterase and Cholesterol Oxidase/Peroxidase: Dissolve in 220 µl Assay Buffer prior to use. If the kit is to be used on multiple occasions rather than all at once, aliquot into convenient portions and store at -20°C.
Cholesterol Standard: (2 µg/µl) Ready to use as supplied. Warm to room temperature before use. Store at 4°C

Cholesterol Assay Protocol:

1. Separation of HDL and LDL/VLDL: Mix 100 µl of Precipitation Buffer with 100 µl of serum sample in a microcentrifuge tube. Incubate 10 min. at room temperature, centrifuge at 2000 X g (~5000 rpm on bench-top microcentrifuge) for 10 min. Transfer supernatant (HDL fraction) to a new tube. The precipitate is the LDL/VLDL fraction. To accurately measure the LDL/VLDL cholesterol, respin the precipitate and carefully remove any traces of HDL containing supernatant. Resuspend precipitate in 200 µl PBS (not provided).

Note: If the supernatant is cloudy, re-centrifuge. If the sample remains cloudy, dilute 1:1 with PBS and repeat the separation procedure. Multiply final results by 4 if diluted or by 2 to correct for dilution. For most accurate results, each sample should have its own background well.

2. Standard Curve: For an absorbance-based assay, **d**ilute the Cholesterol Standard to 0.25 mg/ml by adding 20 µl of the Cholesterol Standard to 140 µl of Assay Buffer, mix well. Transfer 0, 4, 8, 12, 16, 20 µl in duplicate to a series of wells in a 96-well plate. Adjust all well volumes to 50 µl with Assay Buffer to generate 0, 1, 2, 3, 4, 5 µg/well of the Cholesterol Standard. If a fluorimetry based assay is preferred, dilute the standard as described above then dilute the standard another 10X by transferring 20 µl of the diluted standard to 180 µl of Assay buffer to give a 25 µg/ml Standard solution. Transfer 0, 4, 8, 12, 16, 20 µl in duplicate into a series of wells as described above for the absorbance-based assay.

3. Sample Preparation: Use 1 to 20 µl of the HDL or LDL/VLDL fractions prepared previously. Adjust all well volumes to 50 µl with assay buffer. If the expected cholesterol content of the samples is not known, use 2-3 different volumes. Final readings must be within the range established by the standard curve.

4. Reaction Mix: Each reaction requires 50 µl of Reaction Mix. Prepare sufficient Reaction Mix for the total number of wells to be measured.

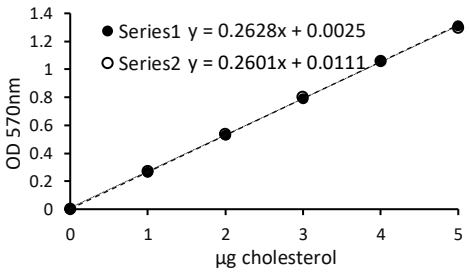
	Absorbance Based Assay		Background Wells	Fluorescence Based Assay		
Assay Buffer:	44 µl	(46 µl)	46 µl	45.6 µl	(47.6 µl)	(*Only use Cholesterol Esterase if Total
ADHP Solution:	2 µl		2 µl	0.4 µl		Cholesterol is to be determined. Omit if
Enzyme Mix	2 µl		-------	2.0 µl		Free Cholesterol only is to be determined)
Cholesterol Esterase*	2 µl	(0 µl)	2 µl (0 µl)	2.0 µl	(0 µl)	

Mix and add 50 µl to each well

5. Measurement: Monitor the reaction progress in a plate reader for 60 min at 37°C using either OD (570 nm) or fluorescence (Ex/Em 538/587 nm).

6. Typical Results:

µg Standard	Standard Raw Values (OD)		Background Corrected Values (OD)	
0	0.0499	0.0488	0	0
1	0.3156	0.3217	0.2657	0.2729
2	0.5795	0.5854	0.5296	0.5366
3	0.8412	0.8528	0.7913	0.8040
4	1.1097	1.1073	1.0598	1.0585
5	1.3608	1.3775	1.3109	1.2957



7. Calculations: Subtract 0 standard reading from all readings. Plot the standard curve. The slope defines the OD/µg. There is a distinct convexity to the absorbance curve for values above about 1 OD. A similar convexity is also seen in fluorescence-based assays. Fitting the data to a 2nd order polynomial will give more accurate results with unknowns than a straight line. Subtract the paired background from each sample. Apply the standard curve slope to background corrected samples to determine the cholesterol content of the unknown samples, then correct for sample dilution due to sample processing.

A. Raw absorbance $_{Test}$ − Background Control $_{Test}$ = Net Absorbance $_{Test}$
B. Net Absorbance / Slope of Standard Curve = µg cholesterol in sample well.
C µg cholesterol in sample well / µl sample added to well X 2= µg cholesterol /µl sample (corrected for dilution during processing)
D. µg cholesterol /µl sample X 100 µl original sample volume = total µg cholesterol in sample

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.

48511 Warm Springs Blvd. # 213, Fremont, CA 94539 +1(408)739-9315 contact@akrivisbio.com