3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

HDL and LDL/VLDL Quantitation Kit

Catalog Number **MAK045**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/ λ_{em} = 587 nm) product, proportional to the cholesterol present. This kit can also be used to determine the concentration of free cholesterol and cholesteryl esters present in a sample. This kit is suitable for use with plasma and serum samples.

Components

The kit is sufficient for 100 assays in 96 well plates.

Cholesterol Assay Buffer Catalog Number MAK045A	25 mL
2× LDL/VLDL Precipitation Buffer Catalog Number MAK045B	10 mL
Cholesterol Probe in DMSO Catalog Number MAK045C	0.2 mL
Enzyme Mix Catalog Number MAK045D	1 vl
Cholesterol Esterase Catalog Number MAK045E	1 vl
Cholesterol Standard, 2 µg/µL Catalog Number MAK045F	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader
- Phospate Buffered Saline (Catalog Number P5368 or equivalent)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Cholesterol Assay Buffer – Allow buffer to come to room temperature before use.

Cholesterol Probe – Warm to room temperature to thaw the solution prior to use. Store protected from light and moisture at –20 °C. Upon thawing, the Cholesterol Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the colorimetric Cholesterol Probe Solution 5 to 10-fold with Cholesterol Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Cholesterol Esterase and Enzyme Mix – Reconstitute each in 220 µL of Cholesterol Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use and protect from light. Use within two months of reconstitution.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Cholesterol Standards for Colorimetric Detection
Dilute 20 µL of the 2 µg/µL Cholesterol Standard Solution with 140 µL of the Cholesterol Assay Buffer to prepare a 0.25 µg/µL standard solution. Add 0, 4, 8, 12, 16, and 20 µL of the 0.25 µg/µL Cholesterol Standard solution into a 96 well plate, generating 0 (blank), 1, 2, 3, 4, and 5 µg/well standards. Add Cholesterol Assay Buffer to each well to bring the volume to 50 µL.

Cholesterol Standards for Fluorometric Detection
Dilute 10 µL of the 2 µg/µL Cholesterol Standard Solution with 790 µL of the Cholesterol Assay Buffer to prepare a 25 ng/µL standard solution. Add 0, 4, 8, 12, 16, and 20 µL of the 25 ng/µL Cholesterol Standard solution into a 96 well plate, generating 0 (blank), 0.1, 0.2, 0.3, 0.4, and 0.5 ng/well standards. Add Cholesterol Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Both the colorimetric and fluorometric assays require 50 µL of sample for each reaction (well).

Separation of HDL and LDL/VLDL: Mix 100 µL of the 2× Precipitation Buffer with 100 µL of the serum sample in a microcentrifuge tube. Incubate for 10 minutes at room temperature and then centrifuge the samples at 2,000 × *g* for 10 minutes. Transfer the supernatant fraction (HDL) to a new tube. The precipitant contains the LDL/VLDL fraction. To measure LDL/VLDL, centrifuge the samples again at 2,000 × *g* for 10 minutes and remove any remaining trace HDL supernatant. Resuspend the precipitate in 200 µL of PBS.
Note: If the supernatant is cloudy, the sample should be recentrifuged. If the sample remains cloudy, dilute the sample 1:1 with PBS and repeat the separation procedure.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Bring samples to a final volume of 50 µL with Cholesterol Assay Buffer.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Total Cholesterol and Standards	Free Cholesterol
Cholesterol Assay Buffer	44 µL	46 µL
Cholesterol Probe	2 µL	2 µL
Cholesterol Enzyme Mix	2 µL	2 µL
Cholesterol Esterase	2 µL	–

Note: Cholesterol Esterase hydrolyzes cholesteryl esters to cholesterol. In the presence of Cholesterol Esterase, the assay detects total cholesterol, both free cholesterol and cholesteryl esters. To detect free cholesterol only, omit the Cholesterol Esterase from the reaction and add 46 µL of the Cholesterol Assay Buffer to the Reaction Mix. To determine cholestryl esters, subtract the free cholesterol value from the total cholesterol value.

The cholesterol standard contains a mixture of free cholesterol and cholesteryl esters. The Reaction Mix containing Cholesterol Esterase must be used in the reactions for the Cholesterol Standards to convert all of each standard to cholesterol.

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at 37 °C. Protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for either assay is the value obtained for the 0 (blank) cholesterol standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate cholesterol standards to plot a standard curve. The amount of cholesterol present in the samples may be determined from the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Cholesterol

$$(S_a/S_v) \times Df^* = C$$

S_a = Amount of cholesterol in unknown sample (μg) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of cholesterol in sample

Df = The dilution factor will be 2 due to the 1:1 dilution with the 2× Precipitation buffer. If the sample requires further dilution with PBS, the factor will need to be adjusted accordingly.

Cholesterol molecular weight: 386.65 g/mole.

Sample Calculation

Amount of Cholesterol (S_a) = 5.84 μg

Sample volume (S_v) = 50 μL

Df = 2

Concentration of cholesterol in sample

$$(5.84\ \mu g/50\ \mu L) \times 2 = 0.2336\ \mu g/\mu L$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

LS,MAM 06/12-1