Technical Bulletin

Cholesterol/Cholesteryl Ester Kit II

Catalog Number MAK396

Product Description

Cholesterol is an essential molecule in all animal life. It has been involved in both normal development and diseases. Most of the cholesterol in blood is in the form of cholesteryl esters. These esters can be hydrolyzed to free cholesterol under the appropriate conditions.

The Cholesterol/Cholesteryl Ester Quantitation Kit provides a simple method for sensitive quantification of free cholesterol, cholesteryl esters, or both using a colorimetric method. In the assay, free cholesterol is oxidized by cholesterol dehydrogenase to generate NADH which reacts with a sensitive probe resulting in strong absorbance at 450 nm. The assay can detect free or total cholesterol, depending upon whether esterase is utilized to hydrolyze cholesterol esters present. Cholesteryl ester can be determined by subtracting the value of free cholesterol from the total cholesterol (cholesterol plus cholesteryl esters). The probe in the kit is stable, sensitive and specific. The assay can tolerate significant interference from various compounds within samples.

The kit is suitable for the quantification of cholesterol/cholesteryl ester in serum, cells, and tissue samples.

Components

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

- Cholesterol Assay Buffer 25 mL
  Catalog Number MAK396A
- Substrate Mix 1 vial
  Catalog Number MAK396B
- Enzyme Mix 1 vial
  Catalog Number MAK396C
- Esterase 1 vial
  Catalog Number MAK396D
- Cholesterol Standard (2 µg/µL) 100 µL
  Catalog Number MAK396E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well flat-bottom plate. It is recommended to use clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Microcentrifuge capable of RCF ≥15,000 × *g*
- Heat block capable of 50 °C
- Vacuum source
- Chloroform (Catalog Number 472476 or equivalent)

Reagents and Equipment Required but Not Provided (continued)

- 2-Propanol (Catalog Number 190764 or equivalent)
- IGEPAL® CA-630(Catalog Number I8896 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at -20 °C, protected from light.

Preparation Instructions

Keep enzymes and cholesterol standard on ice while using.

Cholesterol Assay Buffer: Allow Assay Buffer to warm to room temperature before use.

Substrate Mix: Reconstitute vial with 220 µL of Assay Buffer and mix thoroughly. The solution is stable for two months at 4 °C after reconstitution.

Enzyme Mix: Dissolve vial in 220 µL of Cholesterol Assay Buffer before use. Aliquot and store at -20 °C. Use within two months after reconstitution.

Esterase: Dissolve vial in 220 µL of Cholesterol Assay Buffer before use. Aliquot and store at -20 °C. Use within two months after reconstitution.

Extraction Solution: Combine chloroform, 2-propanol, and IGEPAL® CA-630 (reagents not included) in a 7:11:0.1 ratio. Mix well.

Procedure

Standard Curve Preparation

Note: If both free and total cholesterol determinations are desired, prepare **two** identical standard curves according to Table 1. One set of standards is used for total cholesterol determination and undergoes ester hydrolysis. The second set of standards is used for free cholesterol determination and does not undergo ester hydrolysis.

1. Prepare a 0.25 µg/µL Cholesterol Standard by diluting 20 µL of the Cholesterol Standard (2 µg/µL) with 140 µL of Cholesterol Assay Buffer, mix well. Prepare Cholesterol Standards according to Table 1. Mix well.

Table 1.
Preparation of Cholesterol Standards

Well	0.25 µg/µL Cholesterol Standard	Cholesterol Assay Buffer	Cholesterol (µg /well)
1	0 µL	50 µL	0
2	4 µL	46 µL	1
3	8 µL	42 µL	2
4	12 µL	38 µL	3
5	16 µL	34 µL	4
6	20 µL	30 µL	5

Sample Preparation

1. Dilute serum 10-fold with Cholesterol Assay Buffer and use 2-20 µL for each test.
2. For cells or tissue samples, extract 10^6 cells or 10 mg tissue with 200 µL of Extraction Solution (prepared per instructions in Reagent Preparation section) in a microhomogenizer.
3. Centrifuge the extract for 5 minutes at 15,000 × *g*.
4. Transfer the liquid phase to a new tube and dry in a heat block at 50 °C. After drying, place samples under vacuum for 30 minutes to remove any remaining solvent.

5. Dissolve dried lipids with 200 µL of Cholesterol Assay Buffer by sonicating or vortexing until homogeneous (the solution may be cloudy; this is acceptable).
6. The extraction procedure can be scaled up if larger amounts of sample are desired.
7. Use 1- 50 µL of extract per assay.
8. Adjust total volume to 50 µL/well with Cholesterol Assay Buffer.
9. For unknown samples, test different amounts of sample to ensure that readings are within the Standard Curve range.

Ester Hydrolysis (Total Cholesterol Determination Only)

Notes:

a) Cholesterol Esterase hydrolyzes cholesteryl esters to cholesterol. In the presence of Cholesterol Esterase, the assay detects both free cholesterol and cholesteryl esters. To detect free cholesterol only, omit the Ester Hydrolysis step. To determine Cholesteryl Ester only, subtract the value of free cholesterol from the value of total cholesterol (cholesterol plus cholesteryl esters).

b) The Cholesterol Standard contains a mixture of free cholesterol and cholesterol esters in a similar ratio as for serum. Cholesterol Esterase must be added to the standard reaction to convert all cholesterol in the standard.

1. Add 2 µL of Esterase to each standard and sample well for which the Total Cholesterol value is desired. (See Notes a and b above).
2. Incubate for 30 minutes at 37 °C.

Reaction Mix

1. Mix enough reagents for the number of assays to be performed. For each well, prepare appropriate Reaction Mix according to Table 2 (50 µL for Free Cholesterol and 48 µL for Total Cholesterol). Mix well.

Table 2.
Reaction Mix Preparation

Reagent	**Free Cholesterol Reaction Mix**	**Total Cholesterol Reaction Mix**
Cholesterol Assay Buffer	46 µL	44 µL
Substrate Mix	2 µL	2 µL
Cholesterol Enzyme Mix	2 µL	2 µL

2. Add 50 µL of the Free Cholesterol Reaction Mix to each well containing Standard or Sample for which a Free Cholesterol determination is desired. See note under Standard Curve Preparation.
3. Add 48 µL of the Total Cholesterol Reaction Mix to each well containing Standard or Sample for which a Total Cholesterol determination is desired. See note under Standard Curve Preparation.

Measurement

Incubate plate for 30 minutes at 37 °C, protected from light. Measure absorbance at 450 nm (A_{450}) in a microplate reader.

Results

1. Subtract the 0 Standard background A_{450} reading from all A_{450} readings.
2. Plot the standard curve. Plot separate curves for Free Cholesterol and Total Cholesterol.
3. Apply Sample A_{450} readings to the appropriate standard curve to determine amount of Free or Total Cholesterol.
4. Cholesterol concentration in samples is calculated as follows:

   Cholesterol (μg/μL) = (C/V) × D

where:

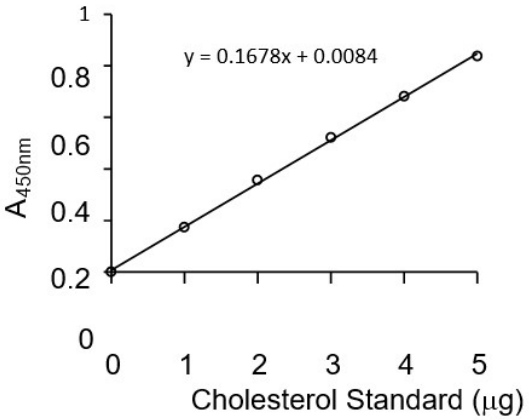
C = Amount of cholesterol determined from Standard Curve (in μg)

V = Volume of sample added into the reaction well (in μL)

D = Sample dilution factor

Cholesterol molecular weight = 386.65.

Figure 1.
Typical Cholesterol Standard Curve. Cholesterol/Cholesteryl Ester was quantified according to the kit protocol. Background from the 0 Standard reading (without cholesterol) was subtracted from all readings.



Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.