Technical Bulletin

Cholesterol Quantification Assay Kit

CS0005

Product Description

Cholesterol is a lipid that constitutes 20-25% of the structural components of cell membranes. Cholesterol also regulates the functions of the transporters and signaling proteins present on the plasma membrane.[1] The major sites of cholesterol synthesis are the small intestine and liver.[2] Cholesterol circulates in the blood as both the free acid and as cholesterol esters. Increased cholesterol levels are associated with the development of atherosclerosis and cardiovascular diseases. Thus, serum cholesterol regulation has an important therapeutic role.

The Cholesterol Quantification Assay Kit provides a simple and quick procedure for measuring free cholesterol, cholesteryl esters, or total cholesterol (which constitutes both free cholesterol and cholesteryl esters). The cholesterol concentration is determined via a coupled enzymatic reaction. This kit allows for either fluorometric or colorimetric detection, to allow maximum flexibility and a large detection range. The linear ranges are as follows:

- Fluorescence assay: 0.1-0.5 µg
- Colorimetric assay: 1-5 µg

This kit does not require weighing. Apart from the Cholesterol Standard, all the reagents are ready-to-use. The kit was tested on cell culture extracts, serum, plasma, and various cholesterol-containing foods. Several publications[3-6] have cited use of this kit in their research protocols.

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.

Components

This kit contains sufficient reagents for 100 colorimetric tests, or 400 fluorometric tests, in 96-well plates.

Component	Component Number	Amount	Cap Color/ Container Information
Assay Buffer	CS0005A	50 mL	White cap/ bottle
Cholesterol Standard	CS0005B	200 µL	Yellow cap/ vial
Probe	CS0005C	200 µL	Brown vial
Enzyme Mix	CS0005D	200 µL	Red cap/ vial
Cholesterol Esterase	CS0005E	200 µL	Green cap/ vial

Component Information

- Assay Buffer (CS0005A): Ready-to-use. Upon thawing, store at 2-8 °C.
- Cholesterol Standard (CS0005B): Contains a 2 µg/µL cholesterol solution. Store at –20 °C.
- Probe (CS0005C): Ready-to-use. Store at –20 °C, protected from light.
- Enzyme Mix (CS0005D): Ready-to-use. Store at –20 °C.
- Cholesterol Esterase (CS0005E): Ready-to-use. Store at –20 °C.

Reagents and Equipment Required, But Not Provided

- 96-well flat-bottom plates:
  - Use black plates for fluorescence assays.
  - Use clear plates for colorimetric assays.
- Fluorescence (λ_{ex} = 535 nm/λ_{em} = 587 nm) or spectrophotometric (570 nm) multiwell plate reader.
- IGEPAL® CA-630, Cat. No. I3021 (for cholesterol extraction from cells, see below)

Storage/Stability

The kit is shipped on dry ice. Upon receipt, store all components at –20 °C, protected from light. Upon thawing, the Assay Buffer should be stored at 2-8 °C. It is recommended to aliquot all other opened unused components, and store at –20 °C. The unopened kit is stable for 2 years as supplied.

Procedure

- All samples and standards should be run in duplicate.
- A fresh set of standards should be prepared for every use.
- Equilibrate all reagents to room temperature before use.
- Briefly centrifuge vials before opening.
- The kit is configured for 96-well flat-bottom plates.
  - Use black plates for fluorescence assays.
  - Use clear plates for colorimetric assays.
- **For convenience, an Excel-based calculation sheet is available on the Product Detail Page.** Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.

Sample preparation

All assays (samples, standards, and blank) require 50 µL of sample for each reaction (well). Therefore, bring the sample volume to 50 µL. When required, samples should be diluted in Assay Buffer. For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Serum and plasma samples can be tested directly, or diluted if required. Oxalate, citrate, or fluoride should be avoided. The serum or plasma should be separated from the red blood cells within 2 hours. Samples are stable for at least 5-7 days in 2-8 °C, or for at least 3 months in –20 °C.

Cells (1×10^6) can be extracted with 200 µL of chloroform:isoporpanol:IGEPAL® CA-630 (7:11:0.1) in a microhomogenizer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material. Transfer the organic phase to a new tube and air dry at 50 °C for ~30 minutes to remove chloroform. Centrifuge samples using a vacuum concentrator until any residue of organic solvent has been fully removed, and a dry pellet is obtained. Dissolve dried lipids with 200 µL of Assay Buffer, and vortex until the mixture is homogenous.

Best results are obtained when samples are freshly prepared. If this is not feasible, samples should be stored at –20 °C.

Standards for colorimetric detection:

1. Dilute the Cholesterol Standard (yellow cap vial) 8-fold with Assay Buffer to a final concentration of 0.25 µg/µL: 20 µL of the Cholesterol Standard solution with 140 µL of Assay Buffer, to prepare a 1× standard solution.
2. Add 0, 4, 8, 12, 16, and 20 µL of the 1× standard solution into a 96-well plate, generating 0 (blank), 1, 2, 3, 4 and 5 µg/well standards. Bring the volume to 50 µL with Assay Buffer (see Table 1):

Table 1. Standards for the colorimetric assay*

1× Standard Volume	Assay Buffer Volume	Final Amount of Cholesterol per Well
0 µL	50 µL	0 µg (blank)
4 µL	46 µL	1 µg
8 µL	42 µL	2 µg
12 µL	38 µL	3 µg
16 µL	34 µL	4 µg
20 µL	30 µL	5 µg

* Work in duplicate

Reaction mix for colorimetric detection:

- Set up the Colorimetric Reaction Mix according to Table 2.
- 50 µL of the Colorimetric Reaction Mix are required for each reaction (well).
- Multiply the volumes in Table 2 according to the number of wells in the assay.

Table 2. Colorimetric Reaction Mix, per one well

Reagent	Total Cholesterol and Standards	Free Cholesterol*
Assay Buffer	44 µL	46 µL
Probe (Brown vial)	2 µL	2 µL
Enzyme Mix (Red cap vial)	2 µL	2 µL
Cholesterol Esterase (Green cap vial)	2 µL	–

* **Note**: Cholesterol Esterase hydrolyzes cholesteryl esters to cholesterol. In the presence of Cholesterol Esterase, the assay detects total cholesterol (which constitutes both free cholesterol and cholesteryl esters). To detect free cholesterol only, omit the Cholesterol Esterase from the reaction and add 46 µL of Assay Buffer to the Colorimetric Reaction Mix (see Table 2). The Reaction Mix containing Cholesterol Esterase must be used in the reactions for the cholesterol standards.

Standards for fluorometric detection:

- Dilute the Cholesterol Standard (yellow cap vial) 80-fold with Assay Buffer to a final concentration of 0.025 µg/µL: 10 µL of the Cholesterol Standard solution with 790 µL of Assay Buffer, to prepare a 1× standard solution.
- Add 0, 4, 8, 12, 16, and 20 µL of the 1× standard solution into a 96-well plate, generating 0.0 (blank), 0.1, 0.2, 0.3, 0.4 and 0.5 µg/well standards.
- Bring the volume to 50 µL with Assay Buffer (see Table 3):

Table 3. Standards for the Fluorometric Assay*

1× Standard Volume	Assay Buffer Volume	Final Amount of Cholesterol per Well
0 µL	50 µL	0.0 µg (blank)
4 µL	46 µL	0.1 µg
8 µL	42 µL	0.2 µg
12 µL	38 µL	0.3 µg
16 µL	34 µL	0.4 µg
20 µL	30 µL	0.5 µg

* Work in duplicate

Reaction mix for fluorescence detection:

- Set up the Fluorometric Reaction Mix according to Table 4.
- 50 µL of the Fluorometric Reaction Mix is required for each reaction (well).
- Multiply the volumes in Table 4 according to the number of wells in the assay.

Table 4. Fluorometric Reaction Mix, per one well

Reagent	Total Cholesterol and Standards	Free Cholesterol*
Assay Buffer	48.5 µL	49 µL
Probe (Brown vial)	0.5 µL	0.5 µL
Enzyme Mix (Red cap vial)	0.5 µL	0.5 µL
Cholesterol Esterase (Green cap vial)	0.5 µL	–

* **Note**: Cholesterol Esterase hydrolyzes cholesteryl esters to cholesterol. In the presence of Cholesterol Esterase, the assay detects total cholesterol (which constitutes both free cholesterol and cholesteryl esters). To detect free cholesterol only, omit the Cholesterol Esterase from the reaction and add 49 µL of Assay Buffer to the Fluorometric Reaction Mix (see Table 4). The Reaction Mix containing Cholesterol Esterase must be used in the reactions for the cholesterol standards.

Assay reaction:

1. Add 50 µL of the appropriate Reaction Mix to each of the standard and sample wells.
2. Mix well using a horizontal shaker or by pipetting.
3. Incubate the reaction for 30 minutes at 37 °C. Measurement up to 60 minutes is possible. Protect the plate from light during the incubation.
4. For colorimetric assays, measure the absorbance at 570 nm (A_{570}).
5. For fluorometric assays, measure fluorescence intensity at λ_{ex} = 535 nm/λ_{em} = 587 nm.

Results

Calculations

- An Excel-based calculation sheet is available at the Product Detail Page. **Use this sheet to calculate the test results.**
- If the Excel-based calculation sheet at the Product Detail Page is not used, calculations should be performed as follows:

1. Subtract the blank value (no standard) from all standards and samples values.
2. Plot the absorbance or fluorescence measured for each standard against the standard amount per well.
3. Determine the linear regression equation, and use it to calculate the cholesterol concentration of the sample:

   [(Sample)/(Sample volume)] × DF = µg/µL cholesterol

   Where:

   Sample = Amount of cholesterol in unknown sample (µg), calculated from the standard curve.

   Sample volume = Sample volume added into the wells (50 µL)

   DF = Sample dilution factor (if sample is not diluted, the DF value is 1)

To determine cholesteryl esters, subtract the free cholesterol value from the total cholesterol value.

References

1. Dietschy, J.M., and Turley S.D., *J. Lipid Res.*, **45(8)**, 1375-1397 (2004).
2. Grummer, R.R., and Carroll, D.J., *J. Anim. Sci.*, **66(12)**, 3160-3173 (1988).
3. Chimalapati, S. *et al.*, *eLife*, **9**, e58057 (2020).
4. Ren, Z. *et al.*, *Mol. Med. Rep.*, **21(6)**, 2475-2483 (2020).
5. Jilcott Pitts, S.B. *et al.*, *J. Nutr.*, **152(1)**, 107-116 (2022).
6. Mustafa, I. *et al.*, *BMC Complement Med. Ther.*, **22(1)**, 23 (2022).

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.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

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

Contact Information

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

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.
© 2022 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

CS0005bul Rev 03/22 NA,LB,GCY,MAM DAA00004