Total Cholesterol and Cholesteryl Ester Assay Kit

Catalog No.: abx295080

Size: 96 tests

Detection Range: 0.12 µmol/L – 30 µmol/L

Sensitivity: 0.12 µmol/L

Storage: Store all components at -20°C. Store the Substrate, Enzyme Reagent A, and Extraction Solution in the dark.

Application: For detection and quantification of Total Cholesterol and Cholesteryl Ester content in serum, plasma, animal tissue homogenates, and cell lysates.

Introduction

Cholesterol is the principle sterol in most animals, and has an important role in regulating the fluidity of cell membranes, as well as acting as a precursor to various molecules in fats and oils. It is transported in the blood as Cholesteryl Ester, an esterified form of Cholesterol that binds to lipoproteins. Cholesterol is predominantly known to the public as an indicator of cardiovascular disease risk, but Cholesterol and Cholesteryl Esters have also been implicated in other diseases – some forms of cancer, for example – where links between elevated levels of Cholesterol in serum and tumor malignancy are still being investigated.

Abbexa's Total Cholesterol and Cholesteryl Ester Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Total Cholesterol and Cholesteryl Ester content. Cholesteryl Ester can be converted to Cholesterol by the enzyme cholesterol esterase. This Cholesterol is then converted into 4-cholestenone and hydrogen peroxide by the enzyme cholesterol oxidase. Hydrogen peroxide combines with provided substrates to produce a fluorescent molecule, with an excitation wavelength of 535 nm and emission wavelength of 590 nm. The intensity of the fluorescence is proportional to the Total Cholesterol and Cholesteryl Ester content, which can then be calculated.

Kit components

1. Black 96-well microplate
2. Assay Buffer: 60 ml
3. Substrate: 0.12 ml
4. Enzyme Reagent A: 0.3 ml
5. Enzyme Reagent B: 0.3 ml
6. Cholesterol Standard Solution: 0.2 ml
7. Extraction Solution: 60 ml
8. Plate sealer: 2

Materials required but not provided

1. Fluorescence microplate reader (excitation: 535 nm, emission: 590 nm)
2. PBS (0.01 M, pH 7.4)
3. Pipette and pipette tips
4. 1.5 ml microcentrifuge tubes
5. Centrifuge
6. Vortex mixer
7. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly.
- **Tissue Homogenates:** Carefully weigh out at least 20 mg of tissue, and wash in ice-cold PBS (0.01 M, pH 7.4). Add into pure Extraction Solution in a ratio of 1 : 9 weight (mg) to volume (µl) (i.e. for 20 mg of tissue homogenate, add into 180 µl of pure Extraction Solution). Homogenize manually, using a mechanical homogenizer in an ice water bath (at 4°C). Centrifuge the resulting homogenate at 10,000 × g for 10 minutes. Take the supernatant, keep on ice, and assay immediately.

  Note: To calculate Total Cholesterol and Cholesteryl Ester content in tissue homogenates using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.
- **Cell Lysates:** Collect at least 1 × 10^6 cells for detection. Wash in PBS (0.01 M, pH 7.4). For each 1 × 10^6 cells, add 200 µl of pure Extraction Solution (this volume can be increased up to 400 µl depending on cell density). Homogenize by ultrasonication at 4°C. Centrifuge the resulting homogenate at 10,000 × g for 10 minutes. Take the supernatant, keep on ice, and assay immediately.

  Note: To calculate Total Cholesterol and Cholesteryl Ester content in cell lysates using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Serum (Human, Rat, Mouse, or Rabbit)	100 – 300
10% Rat liver tissue homogenate	50 – 150
10% Mouse kidney tissue homogenate	50 – 200
10% Rat brain tissue homogenate	200 – 400
10% Rat spleen tissue homogenate	50 – 200
Jukat cells	20 – 50

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Chromogenic Reagent A:** Prepare 50 µl of Chromogenic Reagent A by mixing 45 µl of Assay Buffer, 1 µl of Substrate, 2 µl of Enzyme Reagent A, and 2 µl of Enzyme Reagent B in a fresh tube. Prepare Chromogenic Reagent A just before use, and do not store.
- **Chromogenic Reagent B:** Prepare 50 µl of Chromogenic Reagent B by mixing 47 µl of Assay Buffer, 1 µl of Substrate, and 2 µl of Enzyme Reagent A in a fresh tube. Prepare Chromogenic Reagent B just before use, and do not store.
- **Working Extraction Solution:** Prepare only enough Working Extraction Solution as required by the number of samples tested. To prepare, mix Extraction Solution and Assay Buffer in a ratio of 1 : 99 (for example, to prepare 500 µl of Working Extraction Solution, mix 5 µl of Extraction Solution with 495 µl Assay Buffer). Prepare just before use.
- **Stock (50 µmol/L) Standard Solution:** Dilute 12.5 µl of Cholesterol Standard Solution with 1280 µl of Assay Buffer. Mix well. Prepare the Stock (50 µmol/L) Standard Solution just before use.
- **Standards:** Label 7 tubes with 30 µmol/L, 25 µmol/L, 20 µmol/L, 15 µmol/L, 10 µmol/L, 5 µmol/L, and 2 µmol/L. Add 300 µl, 250 µl, 200 µl, 150 µl, 100 µl, 50 µl, and 20 µl of Stock (50 µmol/L) Standard Solution to the 30 µmol/L, 25 µmol/L, 20 µmol/L, 15 µmol/L, 10 µmol/L, 5 µmol/L, and 2 µmol/L tubes respectively, followed by 200 µl, 250 µl, 300 µl, 350 µl, 400 µl, 450 µl, and 480 µl of Assay Buffer, to prepare Standard Dilutions with concentrations 30 µmol/L, 25 µmol/L, 20 µmol/L, 15 µmol/L, 10 µmol/L, 5 µmol/L, and 2 µmol/L. These volumes are summarized in the following table:

Standard Dilution (µmol/L)	**30**	**25**	**20**	**15**	**10**	**5**	**2**
Stock (50 µmol/L) Standard Solution (µl)	300	250	200	150	100	50	20
Assay Buffer (µl)	200	250	300	350	400	450	480

For the blank, or 0 µmol/L standard, use pure Assay Buffer. The volume of each standard will be 500 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- If the Cholesterol Standard Solution does not dissolve completely, the solution may be heated to 65°C for up to 30 minutes. Mix well until the solution is fully homogenous.
- Strictly avoid repeated freeze-thaw cycles of the Substrate, Enzyme Reagent A, or Enzyme Reagent B.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

Determination of Total Cholesterol Content (Free Cholesterol and Cholesteryl Ester)

1. Mark positions on the black 96-well microplate tubes for each standard, sample, and control. *It is strongly recommended to prepare all wells in duplicate.*
2. Add 50 µl of each standard dilution to the respective standard wells, including the blank.
3. Add 50 µl of sample to the sample wells.
4. Add 50 µl of Working Extraction Solution to the control wells.
5. Add 50 µl of Chromogenic Reagent A to all wells.
6. Mix fully by shaking with a microplate shaker for at least 10 seconds, then immediately cover with a plate sealer and incubate at 37°C for 10 minutes.
7. Measure the fluorescence intensity of each well with a fluorescence microplate reader (excitation wavelength: 535 nm, emission wavelength: 590 nm).

Determination of Free Cholesterol Content

1. Mark positions on the black 96-well microplate tubes for each standard, sample, and control. *It is strongly recommended to prepare all wells in duplicate.*
2. Add 50 µl of each standard dilution to the respective standard wells, including the blank.
3. Add 50 µl of sample to the sample wells.
4. Add 50 µl of Working Extraction Solution to the control wells.
5. Add 50 µl of Chromogenic Reagent B to all wells.
6. Mix fully by shaking with a microplate shaker for at least 10 seconds, then immediately cover with a plate sealer and incubate at 37°C for 10 minutes.
7. Measure the fluorescence intensity of each well with a fluorescence microplate reader (excitation wavelength: 535 nm, emission wavelength: 590 nm).

C. Calculation of Results

Plot the standard curve, using the mean fluorescence of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Total Cholesterol in each sample well can be derived with the following formulae:

1. Serum and Plasma samples:

$$\text{Total Cholesterol (µmol/L)} = \text{F} \times \frac{\text{F}_{\text{Sample}} - \text{F}_{\text{Blank}} - \text{b}}{\text{a}}$$

2. Tissue samples:

$$\text{Total Cholesterol (µmol/g fresh weight)} = \mathrm{F} \times \frac{(\mathrm{F_{Sample}} - \mathrm{F_{Control}} - \mathrm{b}) \times \mathrm{V_{Extraction}}}{\mathrm{a} \times \mathrm{W}}$$

3. Cell lysates:

$$\text{Total Cholesterol (µmol/}10^6\text{ cells)} = \mathrm{F} \times \frac{(\mathrm{F_{Sample}} - \mathrm{F_{Control}} - \mathrm{b}) \times \mathrm{V_{Extraction}}}{\mathrm{a} \times \mathrm{N}}$$

where:

$\mathrm{F_{Sample}}$	Fluorescence intensity of sample
$\mathrm{F_{Blank}}$	Fluorescence intensity of blank
$\mathrm{F_{Control}}$	Fluorescence intensity of control
$\mathrm{V_{Extraction}}$	Volume of Extraction Solution added during the preparation of sample
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
F	The dilution factor of sample
W	The fresh weight of sample (g)
N	Number of cells as a factor of 1 × 10^6 (for example, if 5 × 10^6 cells are tested, N = 5).

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.