Total Cholesterol Assay Kit

Catalog No.: abx298882

Size: 96 tests

Detection Range: 0.29 mmol/L – 25.85 mmol/L

Sensitivity: 0.29 mmol/L

Storage: Store all components at 4°C. Store the Enzyme Reagent in the dark.

Application: For detection and quantification of Total Cholesterol content in serum, plasma, and tissue homogenates.

Introduction

Cholesterol is the principal and most abundant sterol found in animals, and has a diverse range of functions in the body. While cholesterol is known to the general public only as a risk factor in cardiovascular disease, it is essential for life. It modifies and maintains the fluidity of the cell membrane, acts as the chemical precursor for numerous more complex molecules (such as Vitamin D and the steroid hormones), and supports the function of various cell signaling systems.

Abbexa's Total Cholesterol Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Total Cholesterol content. Total Cholesterol consists of both free cholesterol and esterified cholesterols. Esterified cholesterols are converted to free cholesterol via the action of the enzyme cholesterol esterase. The free cholesterols are then broken down by cholesterol oxidase to liberate hydrogen peroxide. Hydrogen peroxide converts the chromogen 4-aminoamylpyridine to form a red-brown quinone compound with an absorbance maximum at 510 nm. The intensity of the color is proportional to the Total Cholesterol content of a sample, which can then be calculated.

Kit components

1. 96-well microplate
2. Enzyme Reagent: 30 ml
3. Standard (5.17 mmol/L): 0.2 ml
4. Plate sealer: 2

Materials required but not provided

1. Microplate reader (510 nm)
2. Double-distilled water
3. 1X PBS (0.01 M, pH 7.4)
4. Anhydrous ethanol
5. Normal saline (0.9% NaCl)
6. Pipette and pipette tips
7. 1.5 ml microcentrifuge tubes
8. Centrifuge
9. Incubator

Protocol

A. Preparation of samples

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -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:** Take fresh blood into a serum separation tube, and stand at 25°C for 30 minutes until clotted. Centrifuge at 2000 × g for 15 minutes at 4°C. Carefully take the uppermost liquid layer, and keep on ice. Assay with 24 hours, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Take fresh blood into a plasma preparation tube, and add anticoagulant. Centrifuge at 1000 × g for 10 minutes at 4°C. Carefully take the uppermost liquid layer and keep on ice. Assay within 24 hours, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Carefully weigh 0.02 g – 1 g of tissue, and wash with ice-cold PBS (0.01 M, pH 7.4). Add into anhydrous ethanol in a ratio of 1:9 weight to volume (i.e. for 1 g of tissue, add 9 ml of anhydrous ethanol). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath. Collect the tissue homogenate, and centrifuge at 10,000 × g for 10 minutes at 4°C. Take the supernatant for detection. Assay within 24 hours.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. The diluent used will depend on the sample type. For serum and plasma, use normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For tissue homogenates, use anhydrous ethanol. Dilute samples into different concentrations then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	1
Mouse serum	1
Rat plasma	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1

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.
- Do not add any reducing compounds, such as ascorbic acid, glutathione, and DTT, to the samples.

B. Assay Procedure

1. Mark positions on the 96-well plate for the standard, the blank, and each sample. *It is strongly recommended to prepare all the wells in duplicate.*
2. Add 2.5 µl of double-distilled water to the blank well.
3. Add 2.5 µl of Standard (5.17 mmol/L) to the standard well.
4. Add 2.5 µl of sample to the corresponding sample wells.
5. Add 250 µl of Enzyme Reagent to all wells. *Add the Enzyme Reagent carefully to prevent the formation of any bubbles.*
6. Tap or shake the plate to mix fully. Cover with a plate sealer and incubate at 37°C for 10 minutes.
7. Measure the OD of each well with a microplate reader at 510 nm.

C. Calculation of Results

The concentration of Total Cholesterol in each sample well can be derived with the following formulae:

1. Serum and Plasma samples:

$$\text{Total Cholesterol (mmol/L)} = F \times \frac{(OD_{Sample} - OD_{Blank}) \times C_{Standard}}{OD_{Standard} - OD_{Blank}}$$

2. Tissue samples:

$$\text{Total Cholesterol (mmol/kg tissue)} = F \times \frac{(OD_{Sample} - OD_{Blank}) \times C_{Standard} \times V_{Tissue}}{(OD_{Standard} - OD_{Blank}) \times m}$$

where:

OD_{Sample}	OD value of sample
$OD_{Standard}$	OD value of standard
OD_{Blank}	OD value of blank
$C_{Standard}$	Concentration of standard (5.17 mmol/L)
m	Weight of tissue sample (g)
V_{Tissue}	Volume of tissue homogenate + homogenization medium (ml)
F	The dilution factor of sample