Total Cholesterol Assay Kit

Catalog No.: abx294115

Size: 100 tests

Detection Range: 0.09 mmol/L – 25.85 mmol/L

Sensitivity: 0.09 mmol/L

Storage: Store all components at 2-8°C for up to 12 months. The Enzyme Working Solution should be stored in the dark.

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

Introduction

Total Cholesterol is the total amount of cholesterol in blood, including low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, free cholesterol and cholesterol esters. Cholesterol esters are formed by cholesterol linking to a fatty acid chain.

Abbexa's Total Cholesterol Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Total Cholesterol. Cholesterol esterase can hydrolyze cholesterol esters to produce cholesterol and free fatty acids . Cholesterol oxidase oxidizes cholesterol to produce Δ4-cholestenone and hydrogen peroxide. In the presence of 4-aminoamylpyridine and phenol, hydrogen peroxide catalyzes peroxidase to produce a produce a red quinone compound with an absorbance maximum at 510 nm. The intensity of the color is proportional to the total cholesterol content, which can then be calculated.

Kit components

1. Enzyme Working Solution: 2 x 60 ml
2. 5.17 mM Cholesterol Standard: 0.5 ml

Materials required but not provided

1. Spectrophotometer (510 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Anhydrous ethanol
6. Micropipette and sterile pipette tips
7. Centrifuge
8. 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. They can be stored at -80°C for up to a month.
- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue and add wash in cold PBS (0.01 M, pH 7.4). For each 20 mg of tissue, add 180 µl of anhydrous ethanol. Homogenize manually or mechanically at 4°C. Centrifuge the homogenate at 10,000 x g for 10 minutes to remove insoluble material. Collect the supernatant, keep on ice, and assay immediately.

  Note: To calculate Total Cholesterol concentration in tissue homogenates using the formula 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 normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) for serum and plasma samples, or anhydrous ethanol for tissue samples. 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.
- Ensure reagents are kept sterile.
- It is recommended to pipette samples onto the walls of the tubes to minimize error due to the low volume.

- When measuring low concentration samples, the sample volume may be increased to 20ul. In this case, standard and blank volumes should also be increased.

B. Assay Procedure

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

1. Set the standard, sample and blank tubes and label accordingly.
2. Add 10 µl of double-distilled water to the 2 ml EP blank tube.
3. Add 10 µl of 5.17 mM Cholesterol Standard to the 2 ml EP standard tube.
4. Add 10 µl of sample to the 2 ml EP sample tube.
5. Add 1000 µl of Enzyme Working Solution to all tubes.
6. Mix fully, then incubate all tubes at 37°C for 10 minutes.
7. Set the spectrophotometer to zero with double-distilled water.
8. Measure the OD of each tube at 510 nm with a 0.5 cm diameter cuvette.

C. Calculation of Results

Plot the standard curve, using the OD 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:

$$\text{Total Cholesterol (mmol/L)} = \frac{\Delta A_1 \times c \times f}{\Delta A_2}$$

2. Tissue samples:

$$\text{Total Cholesterol (mmol/kg wet weight)} = \frac{\Delta A_1 \times c \times f \times V}{\Delta A_2 \times m}$$

where:

ΔA_1	$OD_{sample} - OD_{blank}$
ΔA_2	$OD_{standard} - OD_{blank}$
c	the concentration of standard (5.17 mmol/L).
f	Dilution factor of sample before test.
m	the mass of tissue sample (grams).
V	the volume of the homogenate of tissue samples (ml).

Technical Support

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