Free Cholesterol (FC) Assay Kit

Catalog No.: abx294080

Size: 96 tests

Detection Range: 0.07 mmol/L – 24 mmol/L

Sensitivity: 0.07 mmol/L

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

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

Introduction

Abbexa's Free Cholesterol Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Free Cholesterol content. Free Cholesterol in samples is converted to 4-cholestenone + H_2O_2 by Cholesterol Oxidase. In the presence of 4-aminoamylpyridine and Phenol, H_2O_2 is converted to red-colored quinone compounds by Peroxidase. The red compounds have an absorbance maximum at 510 nm. The intensity of the color is proportional to the Free Cholesterol content, which can then be calculated.

Kit components

1. 96-well microplate
2. Enzyme Working Solution: 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. Normal Saline (0.85 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Ethanol (anhydrous)
6. Pipette and pipette tips
7. 1.5 ml microcentrifuge tubes
8. Centrifuge
9. Vortex mixer
10. 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/Plasma:** Serum/Plasma samples can be tested directly.
- **Tissue Homogenates:** Carefully weigh 20 mg of tissue, wash with cold PBS (0.01 M, pH 7.4), then add into 180 µl anhydrous ethanol. Homogenize gently, using a Dounce homogenizer or equivalent, at 4°C. Centrifuge at 10,000 × g for 10 minutes, then collect the supernatant and store on ice for detection.

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 (0.85 % NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human plasa	1
Human serum	1
Rat serum	1
Mouse serum	1
Rabbit serum	1
10 % Rat kidney tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

2. Reagents

Bring all reagents to room temperature before use.

B. Assay Procedure

1. Add 5 µl of Standard into standard wells.
2. Add 5 µl of sample into sample wells.
3. Add 5 µl of double distilled water into blank wells.
4. Add 250 µl of Enzyme Working Solution to each well and mix thoroughly.
5. Incubate at 37°C for 10 minutes, then measure the OD of each well with a microplate reader at 510 nm.

C. Calculation of Results

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

1. Serum and Plasma samples:

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

2. Tissue samples:

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

where:

ΔA_1	$OD_{Sample} - OD_{Blank}$
ΔA_2	$OD_{Standard} - OD_{Blank}$
c	Concentration of the standard (5.17 mmol/L)
f	Sample dilution factor
m	Weight of tissue sample used (g)
V	Volume of anhydrous ethanol used (ml)