AF Cholesterol Assay Kit (Colorimetric/Fluorometric)

LS-K303-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Introduction

CHOLESTEROL is a sterol and lipid present in the cell membranes, and is transported in the bloodstream of all animals. It is used to form cell membranes and hormones, and plays important roles in cell signaling processes. Elevated levels (hypercholesterolemia) have been associated with cardiovascular diseases such as atherosclerosis; whereas, low levels (hypocholesterolemia) may be linked to depression, cancer and cerebral hemorrhage. Simple, direct and automation-ready procedures for measuring cholesterol are very desirable. LSBio's Cholesterol Assay uses a single Working Reagent that combines cholesterol ester hydrolysis, oxidation and color reaction in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at λem/ex = 585/530nm is directly proportional to total cholesterol concentration in the sample.

Key Features

Sensitive and accurate. Linear detection range in 96-well plate: 0.1 to 10 mg/dL cholesterol for colorimetric assays and 0.02 to 2 mg/dL for fluorometric assays.

Convenient. Room temperature assay. No 37°C heater is needed.

High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Applications

Direct Assays: cholesterol in serum, plasma, and other biological samples.

Pharmacology: effects of drugs on cholesterol metabolism.

Components

Component	K303-100
	100 Tests
Assay Buffer	20 mL
Enzyme Mix	120 µL
Dye Reagent	120 µL
Standard	1 mL 300 mg/dL cholesterol

Materials Not Supplied

Pipetting (multi-channel) devices, 96-well plate and plate reader.

Storage

The kit is shipped on ice. Store all kit components at -20 °C. Shelf life: 12 months after receipt.

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Assay Procedure

COLORMETRIC PROCEDURE

Important: bring all reagents to room temperature prior to assay. Serum and plasma samples should be clear and free of turbidity or precipitates. If present, precipitates should be removed by filtration or centrifugation. If not assayed immediately, samples can be stored at -20 to -80°C for at least one year.

1. Standard Curve. Prepare a 10 mg/dL standard (STD) by mixing 15 µL 300 mg/dL Standard and 435 µL Assay Buffer. Further dilute standard (STD) in Assay Buffer as shown below.

No	STD + Assay Buffer	Vol (µL)	Concentration (mg/dL)
1	100µL + 0µL	100	10
2	80µL + 20µL	100	8
3	60µL + 40µL	100	6
4	40µL + 60µL	100	4
5	30µL + 70µL	100	3
6	20µL + 80µL	100	2
7	10µL + 90µL	100	1
8	0µL + 100µL	100	0

Transfer 50 µL diluted standards into wells of a clear 96-well plate. Samples: Transfer 50 µL diluted sample in separate wells.

2. For each reaction well, mix 55 µL Assay Buffer with 1 µL Enzyme Mix and 1 µL Dye Reagent. Add 50 µL of this Working Reagent to each standard and sample well. Tap plate to mix well.
3. Incubate 30 min at room temperature. Read OD at 570 nm.

Subtract blank OD (water, #8) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The cholesterol concentration of Sample is calculated as

$$[\text{Cholesterol}] = \frac{OD_{SAMPLE} - OD_{H_2O}}{\text{Slope}} \times n \ (\text{mg/dL})$$

Where *n* is the dilution factor.

FLUOROMETRIC PROCEDURE

For fluorometric assays, the linear detection range is 0.02 to 2 mg/dL cholesterol.

1. Dilute the Standards prepared in Colorimetric Procedure 1:10 in Assay Buffer.
2. Transfer 50 µL standards and 50 µL samples into separate wells of a black 96-well plate.
3. Add 50 µL Working Reagent (see Colorimetric Procedure). Tap plate to mix.
4. Incubate 30 min at room temperature and read fluorescence at λex = 530nm and λem = 585nm.

If assays in 384-well plate are desired, use 5µL Standards / samples and 45 µL Working Reagent.

The cholesterol concentration of Sample is calculated as

$$[\text{Cholesterol}] = \frac{F_{SAMPLE} - F_{H_2O}}{\text{Slope}} \times n \ (\text{mg/dL})$$

Where *n* is the dilution factor.

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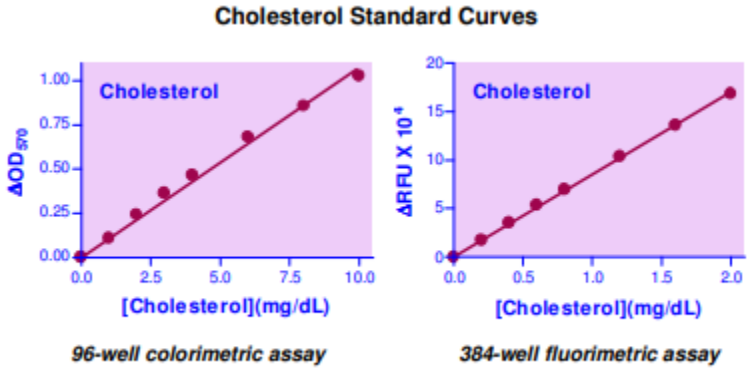
Sample Data



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