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User's Manual and Instructions

AF Cholesterol Assay Kit (Z5030061)

Quantitative Colorimetric/Fluorimetric Determination of Cholesterol

DESCRIPTION

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. BioChain'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.

APPLICATIONS

Direct Assays: cholesterol in serum, plasma, and other biological samples.
Pharmacology: effects of drugs on cholesterol metabolism.

KEY FEATURES

Sensitive and accurate. Linear detection range in 96-well plate: 1 to 100 mg/dL cholesterol for colorimetric assays and 0.2 to 10 mg/dL for fluorimetric 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.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer: 20 mL **Enzyme Mix: 120 uL**
Dye Reagent: 120 µL **Standard: 1 mL 300 mg/dL cholesterol**

Storage conditions. Store reagents at -20°C. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

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-fold diluted 100 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)	10 x Conc. (mg/dL)
1	100µL + 0µL	100	100
2	80µL + 20µL	100	80
3	60µL + 40µL	100	60
4	40µL + 60µL	100	40
5	30µL + 70µL	100	30
6	20µL + 80µL	100	20
7	10µL + 90µL	100	10
8	0µL + 100µL	100	0

Transfer 50 µL diluted standards into wells of a *clear* 96-well plate.

Samples: dilute samples 10-fold (e.g. 10 µL sample with 90 µL Assay Buffer). 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.

Note: if the Sample OD is higher than the Standard OD at 100 mg/dL, dilute sample in assay buffer and repeat the assay. Multiply result by the dilution factor.

CALCULATION

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

$$[\text{Cholesterol}] = \frac{OD_{Sample} - OD_{Blank}}{\text{Slope}} (\text{mg/dL})$$

Note: since both the standards and samples were diluted 10-fold, no dilution factor is required.

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 0.2 to 10 mg/dL cholesterol. Dilute the Standards prepared in Colorimetric Procedure 1:10 in Assay Buffer.

Transfer 50 µL standards and 50 µL samples into separate wells of a *black* 96-well plate.

Add 50 µL Working Reagent (see *Colorimetric Procedure*). Tap plate to mix.

Incubate 30 min at room temperature and read fluorescence at λ_{ex} = 530nm and λ_{em} = 585nm.

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

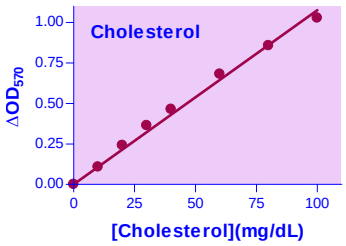
The cholesterol concentration of Sample is calculated as

$$[\text{Cholesterol}] = \frac{F_{Sample} - F_{Blank}}{\text{Slope}} (\text{mg/dL})$$

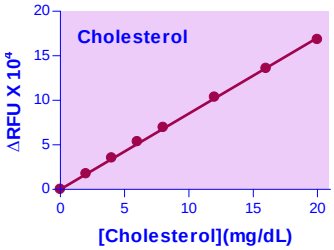
MATERIALS REQUIRED, BUT NOT PROVIDED

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

Cholesterol Standard Curves



96-well colorimetric assay



384-well fluorimetric assay

LITERATURE

[1]. Kayamori, Y. et al (1999) Endpoint Colorimetric Method for Assaying Total Cholesterol in Serum with Cholesterol Dehydrogenase. Clin. Chem. 45: 2158-2163.
[2]. Sundvall J, Leiviska J, Alfthan G, Vartiainen E. (2007). Serum cholesterol during 27 years: assessment of systematic error and affecting factors and their role in interpreting population trends. Clin Chim Acta. 378:93-98.
[3]. Demacker PN, Hijmans AG, van Sommeren-Zondag DF, Jansen AP. (1982). Stability of frozen liquid control sera for assay of cholesterol in high-density lipoprotein. Clin Chem. 28:155-157.