

For Research Use Only. Not For Use In Diagnostic Procedures

Version 2.0

胆固醇检测试剂盒

Cholesterol Assay Kit

Cat.No.MCL3586

Size : 100 tests

Technical literature is available at: www.mesgenbio.com

E-mail MesGen Technical Services if you have questions on use of this system: tech@mesgenbio.com

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. MesGen Biotechnology' 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 lem/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: 0.4 to 100 µg/mL cholesterol for colorimetric assays and 0.1 to 10 µg/mL 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)

Reagent A : 5 mL Reagent B : 3 mL

Reagent C : 2 mL Reagent D : 200 µL

Standard: 1.5 mL 1 mg/mL cholesterol (in Isopropanol)

Do not eat Store at -20° C & in the dark.



Colorimetric 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.

1. Standard Curve. Prepare a 100 µg/mL standard (STD) by mixing 100 µL 2 mg/L Standard and 900 µL dH₂O. Further dilute standard (STD) in dH₂O as shown below. Notice : due to poor solubility of Cholesterol in water, Please mix well when draw dH₂O to Cholesterol STD.

No.	STD + dH ₂ O	Vol (µL)	Concentration (µg/mL)
1	100 µL + 0 µL	100	100
2	80 µL + 20 µL	100	80
3	50 µL + 50 µL	100	50
4	20 µL + 80 µL	100	20
5	10 µL + 90 µL	100	10
6	5 µL + 95 µL	100	5
7	2 µL + 98 µL	100	2
8	1 µL + 99 µL	100	1
9	0 µL + 100 µL	100	0

Transfer 20 µL diluted standards and 20 µL diluted sample into wells of a clear 96-well plate.

2. For each reaction well, mix 50 µL Reagent A, 30 µL Reagent B, 20 µL Reagent C and 2 µL Reagent D. Add 100 µL of this Working Reagent to each standard and sample well. Tap plate to mix well.
3. Incubate 15-20 min at room temperature. Read OD at 570 nm.

Calculation

Subtract blank OD 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

$$[\text{Cholesterol}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{H}_2\text{O}}}{\text{Slope}} \times n$$

n is the dilution factor (generally 10-30 for blood samples).

Note: If the Sample OD is higher than the Standard OD at 100 µg/mL, dilute sample in assay buffer and repeat the assay.

Fluorimetric Procedure

1. Dilute the Standards prepared in Colorimetric Procedure 1:10 in dH₂O.
2. Transfer 20 μL standards and 20 μL samples into separate wells of a black 96-well plate.
3. Add 100 μL Working Reagent (see Colorimetric Procedure). Tap plate to mix.
4. Incubate 15-20 min at room temperature and read fluorescence at $\lambda_{ex} = 530\text{nm}$ and $\lambda_{em} = 585\text{nm}$. If assays in 384-well plate are desired, use 8.5 μL Standards / samples and 41.5 μL Working Reagent. The cholesterol concentration of Sample is calculated as

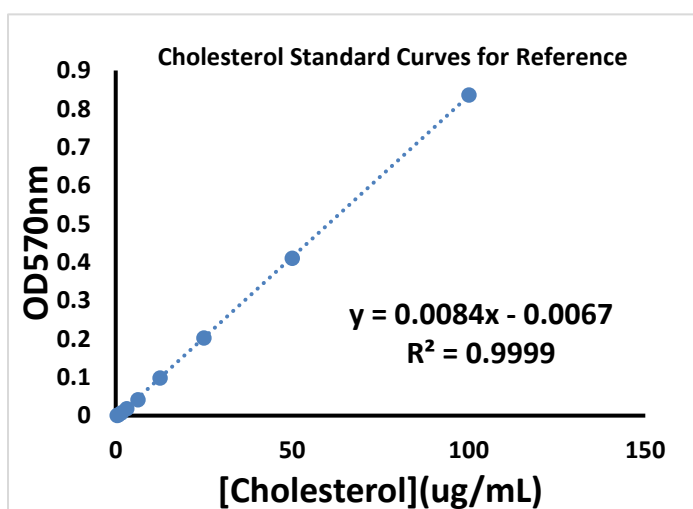
$$[\text{Cholesterol}] = \frac{F_{\text{SAMPLE}} - F_{\text{H}_2\text{O}}}{\text{Slope}} \times n$$

Storage conditions

The kit is shipped on dry ice. Store reagents at -20°C.

Shelf life

6 months after receipt.



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