Technical Bulletin

AF Cholesterol Assay Kit

Catalog Number MAK436

Product 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 of cholesterol in serum (hypercholesterolemia) have been associated with cardiovascular diseases such as atherosclerosis, whereas low cholesterol levels (hypocholesterolemia) may be linked to depression, cancer, and cerebral hemorrhage.

Simple, direct, and automation-ready procedures for measuring cholesterol are very desirable. The AF Cholesterol Assay Kit 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 570 nm or fluorescence intensity at λ_{Ex} = 530 nm/ λ_{Em} = 585 nm is directly proportional to the total cholesterol concentration in the sample. The linear detection range for the assay method is 0.1 – 10 mg/dL by colorimetric detection or 0.02 – 2 mg/dL by fluorometric detection.

The AF Cholesterol Assay Kit is suitable for the quantitative determination of cholesterol and evaluation of drug effects on cholesterol metabolism in serum, plasma, etc.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 20 mL
  Catalog Number MAK436A
- Enzyme Mix 120 µL
  Catalog Number MAK436B
- Dye Reagent 120 µL
  Catalog Number MAK436C
- Standard (300 mg/dL Cholesterol) 1 mL
  Catalog Number MAK436D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are **not** recommended.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Procedure

Sample Preparation

1. Serum and plasma samples should be clear and free of turbidity or precipitates. If present, precipitates should be removed by filtration using a 10 kDa filter or centrifuged for 5-10 minutes at 10,000 × g. If not assayed immediately, samples can be stored at -20 to -80 °C for at least one year.
2. For unknown samples, it is recommended to test several dilutions of sample to ensure the readings are within the linear range. A dilution of 10-fold to 30-fold is typically required for serum and plasma samples.
3. Transfer 50 µL of Sample into separate wells of a 96-well plate (clear for colorimetric assay, black for fluorometric assay). If using a 384-well plate, use 5 µL of Sample per well.

Colorimetric Standard Curve Preparation

1. Prepare a 10 mg/dL Cholesterol Standard by mixing 15 µL of the 300 mg/dL Cholesterol Standard with 435 µL of Assay Buffer.
2. Prepare Cholesterol standards in 1.5 mL microcentrifuge tubes according to Table 2.

Table 2.
Preparation of Colorimetric Cholesterol Standards

Well	10 mg/dL Cholesterol Standard	Assay Buffer	Cholesterol (mg/dL)
1	100 µL	-	10
2	80 µL	20 µL	8
3	60 µL	40 µL	6
4	40 µL	60 µL	4
5	30 µL	70 µL	3
6	20 µL	80 µL	2
7	10 µL	90 µL	1
8	-	100 µL	0

3. Mix well and transfer 50 µL of each Standard into separate wells of a clear 96-well plate. If using a 384-well plate, use 5 µL of each Standard per well.

Fluorometric Standard Curve Preparation

1. Prepare a 2 mg/dL Cholesterol Standard by mixing 5 µL of the 300 mg/dL Cholesterol Standard with 745 µL of Assay Buffer. Prepare Cholesterol standards in 1.5 mL microcentrifuge tubes according to Table 3.

Table 3.
Preparation of Fluorometric Cholesterol Standards

Well	2 mg/dL Cholesterol Standard	Assay Buffer	Cholesterol (mg/dL)
1	100 µL	-	2.0
2	80 µL	20 µL	1.6
3	60 µL	40 µL	1.2
4	40 µL	60 µL	0.8
5	30 µL	70 µL	0.6
6	20 µL	80 µL	0.4
7	10 µL	90 µL	0.2
8	-	100 µL	0

2. Mix well and transfer 50 µL of each Standard into separate wells of a black 96-well plate. If using a 384-well plate, use 5 µL of each Standard per well.

Working Reagents

Mix enough reagents for the number of assays to be performed. For each Standard and Sample well, prepare 57 µL of Working Reagent according to Table 4.

Table 4.
Preparation of Working Reagent

Reagent	**Working Reagent**
Assay Buffer	55 µL
Enzyme Mix	1 µL
Dye Reagent	1 µL

Measurement

1. Add 50 µL of Working Reagent to each Standard and Sample well. If using a 384-well plate, use 45 µL of Working Reagent.
2. Mix well.
3. Incubate the plate for 30 minutes at room temperature.
4. Measure the optical density at 570 nm (OD_{570}) or fluorescence (RFU) at λ_{Ex} = 530 nm/λ_{Em} = 585 nm.
5. If the sample reading is higher than the 10 mg/dL standard reading in the Colorimetric Assay or the 1 mg/dL standard reading in the Fluorometric Assay, dilute sample in Assay Buffer and repeat the assay.

Results

1. Subtract the 0 Standard reading from all Standard readings.
2. Plot the corrected OD_{570} or RFU Standard readings against the Standard concentrations. Determine the slope of the Standard curve.
3. Calculate the cholesterol concentration of Sample:

$$\text{Cholesterol (mg/dL)} = \frac{R_{Sample} - R_{Blank}}{\text{Slope}} \times DF$$

where

R_{Sample} = Optical density (OD_{570}) or fluorescence intensity (RFU) reading of the Sample

R_{Blank} = Optical density (OD_{570}) or fluorescence intensity (RFU) reading of the Blank (Standard #8)

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Figure 1.
Typical Cholesterol Colorimetric Standard Curve (96-well format).

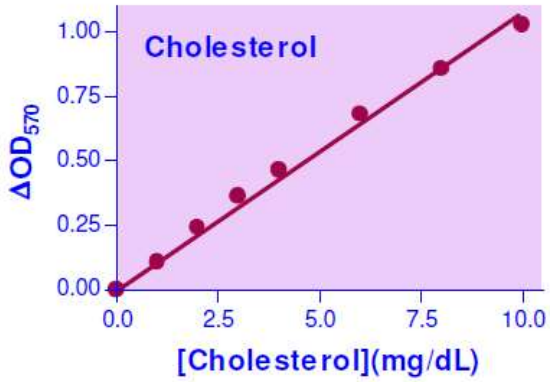
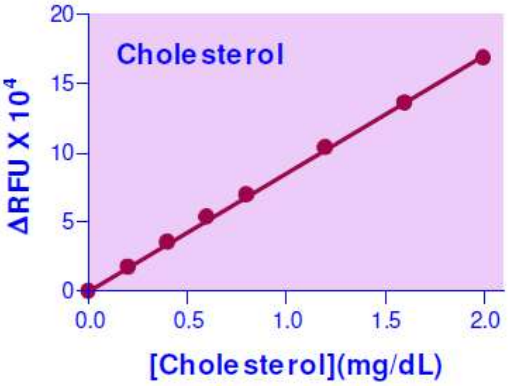


Figure 2.
Typical Cholesterol Fluorometric Standard Curve (384-well format).



References

1. Chappuis, E., et al., Alpha-galacto-oligosaccharides at low dose improve liver steatosis in a high-fat diet mouse model. *Molecules*, **22(10)**, 1725 (2017).
2. Gallagher, A.J., et al., (2017). Energy metabolism in mobile, wild-sampled sharks inferred by plasma lipids. *Conservation physiology*, **5(1)**: cox002 (2017).
3. Lee, S.M., et al., GCG-rich tea catechins are effective in lowering cholesterol and triglyceride concentrations in hyperlipidemic rats. *Lipids*, **43(5)**: 419-429 (2008).

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