# EnzyChrom™ AF Cholesterol Assay Kit (E2CH-100)

## 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. BioAssay Systems' EnzyChrom™ 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: 0.1 to 10 mg/dL cholesterol for colorimetric assays and 0.02 to 2 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 µL**
**Dye Reagent: 120 µL** **Standard: 1 mL 300 mg/dL cholesterol**

**Storage conditions**. The kit is shipped on ice. Store reagents at -20°C. Shelf life: 12 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 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.

## 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

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

*n* is the dilution factor (generally 10-30 for blood samples).
**Note:** If the Sample OD is higher than the Standard OD at 10 mg/dL, dilute sample in assay buffer and repeat the assay.

## FLUORIMETRIC PROCEDURE

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 $\lambda_{ex}$ = 530nm and $\lambda_{em}$ = 585nm.

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

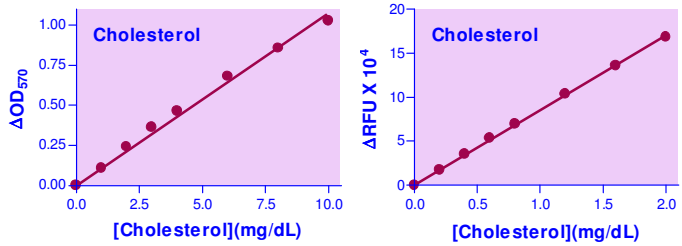
The cholesterol concentration of Sample is calculated as

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

## 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***

# PUBLICATIONS

[1]. Chappuis, E., Morel-Depeisse, F., Bariohay, B., & Roux, J. (2017). Alpha-galacto-oligosaccharides at low dose improve liver steatosis in a high-fat diet mouse model. Molecules, 22(10), 1725.
[2]. Gallagher, A. J., Skubel, R. A., Pethybridge, H. R., & Hammerschlag, N. (2017). Energy metabolism in mobile, wild-sampled sharks inferred by plasma lipids. Conservation physiology 5(1): cox002.
[3]. Lee, SM et al (2008).GCG-rich tea catechins are effective in lowering cholesterol and triglyceride concentrations in hyperlipidemic rats. Lipids 43(5): 419-429.