EnzyChrom™ Cholesterol Assay Kit (ECCH-100)

Quantitative Colorimetric Determination of Cholesterol at 340 nm

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 is based on cholesterol esterase hydrolysis of cholesterol esters to form free cholesterol and cholesterol dehydrogenase catalyzed conversion of cholesterol to cholest-4-ene-3-one, in which NAD is reduced to NADH. The optical density of the formed NADH at 340 nm is directly proportionate to the 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. Detection limit of 5 mg/dL, linearity up to 300 mg/dL cholesterol in 96-well plate assay.
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**
NAD Solution: 2 x 1 mL **Standard: 1 mL 300mg/dL cholesterol**

Storage conditions. The kit is shipped on ice. 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.

PROCEDURES

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 in a table centrifuge. 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 standard (STD) by mixing 40 µL 300 mg/dL Standard and 360 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	300
2	80µL + 20µL	100	240
3	60µL + 40µL	100	180
4	40µL + 60µL	100	120
5	30µL + 70µL	100	90
6	20µL + 80µL	100	60
7	10µL + 90µL	100	30
8	0µL + 100µL	100	0

Transfer 50 µL diluted standards into wells of the 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. Prepare enough NAD solution in Assay Buffer as follows: for each reaction well, mix 40 µL Assay Buffer with 18 µL the provided NAD Solution.

   Add 50 µL of diluted NAD to standards and sample wells. Tap plate to mix well.

   Let stand 5 min at room temperature. Read background optical density at 340nm (OD_o).

3. Prepare enough enzyme mix as follows: for each reaction well, mix 10 µL Assay Buffer with 1 µL provided Enzyme Mix. Add 10 µL diluted enzyme mix per well. Tap plate to mix thoroughly. Note: the enzyme mix may appear to be turbid, but will be clear after mixing into the reaction mixture.

4. Incubate 30 min at room temperature. Read OD_{30} at 340nm.

5. *Calculation.* Subtract OD_0 from OD_{30} for the standard and sample wells. Use the ΔOD values to determine sample cholesterol concentration from the standard curve. ***Note: since both the standards and samples were diluted 10-fold, no dilution factor is required.***

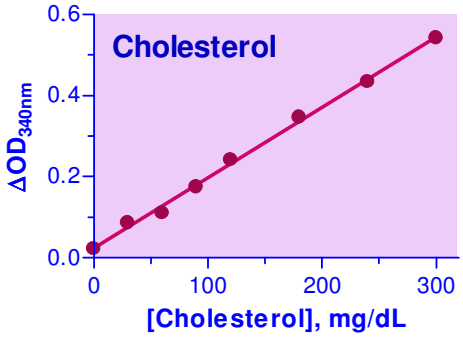
Note: if the sample OD value is higher than OD for the 300 mg/dL standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

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

EXAMPLES

Samples were run in duplicate according to the standard procedure. The cholesterol concentrations (mg/dL) were 105 ± 3 for a human serum, 155 ± 11 for human plasma, 157 ± 2 for a bovine serum, 68 ± 2 for a rat serum, 129 ± 3 for a mouse serum, 123 ± 2 for a goat serum sample.



Standard Curve in 96-well plate assay

PUBLICATIONS

[1]. Lee, S.M. et al (2008). GCG-Rich Tea Catechins are Effective in Lowering Cholesterol and Triglyceride Concentrations in Hyperlipidemic Rats. Lipids 43: 419-429.

[2]. Khan, M.A. et al (2009). Statins impair CD1d-mediated antigen presentation through the inhibition of prenylation. J Immunol 182(8): 4744-4750.

[3]. Mellado, M. et al (2008). Rough agave flowers as a potential feed resource for growing goats. Rangeland Ecol Manage 61: 640-646.