Cholesterol Assay Kit

Cholesterol Assay Kit is a detection kit for the quantification of Cholesterol in serum and plasma.

Catalog number: ARG81630

Package: 100 tests

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INTRODUCTION

Cholesterol is an organic molecule. It is a sterol (or modified steroid), a type of lipid. Cholesterol is biosynthesized by all animal cells and is an essential structural component of animal cell membranes. It is a yellowish crystalline solid.

Cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acid and vitamin D. Cholesterol is the principal sterol synthesized by all animals. In vertebrates, hepatic cells typically produce the greatest amounts. It is absent among prokaryotes (bacteria and archaea), although there are some exceptions, such as Mycoplasma, which require cholesterol for growth. [Provide by Wikipedia: Cholesterol]

PRINCIPLE OF THE ASSAY

This Cholesterol Assay Kit is a simple colorimetric assay that measures the concentrations of Cholesterol in serum and plasma samples. This kit 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 O.D. 340 nm is directly proportionate to the cholesterol concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	20 mL	-20°C
Enzyme Mix	120 µL	-20°C
NAD Solution	1 mL x 2	-20°C
Standard (300 mg/dL cholesterol)	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 340 nm
- Clear flat-bottom 96 well microplate
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collection the supernatant and dilute 10-fold (E.g., 10 µL of sample with 90 µL of Assay Buffer) for assay.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collection the supernatant and dilute 10-fold (E.g., 10 µL of sample with 90 µL of Assay Buffer) for assay.

Note:

- 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.

REAGENT PREPARATION

- **Working NAD Solution:** for each well, mixing 40 µL of Assay Buffer with 18 µL of NAD Solution.
- **Working Enzyme Mix:** for each well, mixing 10 µL of Assay Buffer with 1 µL of Enzyme Mix.
- **Standard:** mixing 40 µL of 300 mg/dL Standard and 360 µL of Assay Buffer. Dilute Standards in Assay Buffer as follow:

Standard tube	10X Conc. (µM)	Assay Buffer (µL)	Standard 30 mg/dL (µL)
S1	300	0	100
S2	240	20	80
S3	180	40	60
S4	120	60	40
S5	90	70	30
S6	60	80	20
S7	30	90	10
S0	0	100	0

Note: Since both each Standard and sample were diluted 10-fold, the final conc. should be 10X.

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge tubes before use.

	Standard well	Sample well
Each diluted Standard	50 µL	
Each Sample		50 µL
Working NAD Solution	50 µL	50 µL
Tap plate to mix briefly. Incubate for **5 minutes** at **room temperature**.		
Read the absorbance at **O.D. 340 nm**. **(OD_0)**		
Working Enzyme Mix	10 µL	10 µL
Tap plate to mix briefly. Incubate for **30 minutes** at **room temperature**.		
Read the absorbance at **O.D. 340 nm**. **(OD_{30})**		

Note: The Working Enzyme Mix may appear to be turbid, but will be clear after mixing into the reaction mixture.

QUALITY ASSURANCE

Sensitivity

5 mg/dL

CALCULATION OF RESULTS

1. Subtract OD_0 from OD_{30} for each Standard and Sample. Use the ΔOD value 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.

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Cholesterol Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.