OTTO Scientific™

TOTAL CHOLESTEROL Assay Kit

Cat.No: OttoBC135

Summary & Explanation

Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is syn- thesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for athero- sclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders. Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889. In the Liebermann-Burchard reaction, cholesterol forms a bluegreen dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/ concentrated sulfuric acid medium. The Abell and Kendall method is specific for cholesterol, but is technically complex and requires the use of corrosive reagents. In 1974, Roeschlau and Allain described the first fully enzymatic method. This method is based on the determination of A 4 cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed. Optimization of ester cleavage (>99.5%) allows standardization using primary and secondary standards and a direct comparison with the CDC and NIST reference methods. The Analyticon cholesterol assay meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3% for both precision and bias.

Test Principle

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to A4 - Cholestenone and hydrogen peroxide. Hydrogen peroxide created forms a red dyestuff by reacting with 4-aminoantipyrine and phenol under the catalytic action of peroxidase. The color intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.

Cholesterol ester + H2O ⟶ Cholesterol + fatty acid

Cholesterol + O2 ⟶ Cholesten-3-on + H2O2

H2O2 + phenol + 4-aminoantipyrine ⟶ quinoneimine dye+4 H2O

Kit Compenents

Content	Explanation	Shelf life
Reagent-1	1x30ml	6 months
Calibrator	1x0.5ml	6 months
Q.Control	1x0.5ml	6 months

Storage & Stability

Reagent: Stable up to expiry date when stored capped and at +4°C even after start using

Calibration and Quality Control: Reconstitute the contents of with 0.5 ml of redistilled water . Stable for 2 days when stored at +4°C

Reactivity

Universal

Specimen

Serum (Edta or Heparin)
Heparinized or EDTA plasma. Do not use citrate, oxalate or fluorideplasma.
Stability: 7 days at +2°C - +8°C, 30 days at - 70°C

Assay Range

30 - 800 mg/dl

Refference Range

Each laboratory is recommended to establish their own reference values.

Analytical Performance

Inter Assay Coefficent of Variation (CV) % 2.7
Intra Assay Coefficent of Variation (CV) % 2.8

Procedure

Wavelength	540nm (±10nm)
Sample or Standard	3µl
Reagent-1	300µl
Zero Adjustment	Reagent blank

Mix and incubate 5 minutes. Read the absorbance against blank within 30 minutes.

Calculation

$$\frac{\text{Abs. Samples}}{\text{Abs. Standard}} \times \text{Calibrator conc} = \text{Cholesterol in mg/dl}$$

Warning

For in vitro use only
Do not pipette by mouth
Do not use reagents beyond the expiry date.

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