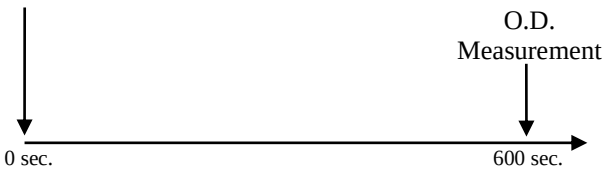
Bioway Chemistry Reagent Series
Cholesterol Test Kit

Detection of Cholesterol in Human Serum or Plasma on Chemistry Analyzers

Cat. No. R018K11 ***CHOL Reagent Kit***

SUMMARY OF TEST PROCEDURE

Figure 1: Procedure Diagram



*Refer to Figure 1 and the package insert for detail

Table 1: Instrument Parameters*

Calibration method	2-point Linear	Slope of reaction	Increase
Testing wavelength	Dλ : 520 nm Sλ : 600 nm	Sample volume	3 µl
Test method	1 point end	R1 volume	300 µl
Reaction temperature	37℃		

INTENDED USE

Bioway Chemistry Reagent Series **CHOL Reagent Kit** (the Kit) is an assay intended for *in vitro* quantitative detection of Cholesterol in human serum or plasma on automated clinical chemistry analyzers.

SUMMARY AND EXPLANATION

Cholesterol is an essential precursor for vitamin D and many steroid hormones synthesis. It is produced in many tissues, including liver and intestine, and then released and circulating to blood and brain tissue. There is a confirmed correlation between elevated cholesterol and many chronic diseases. Excessive serum Cholesterol concentration may due to the lipid and lipoprotein metabolism disorders and causes coronary or peripheral artery diseases. Diagnosing hyperlipoproteinemias is usually based on the result of cholesterol detection. Sometimes the patient with hyperthyroidism or severe liver diseases may show the symptom of depressed serum cholesterol level.

TEST PRINCIPLES

The Kit utilizes CHOD-PAP method to measure the amount of CHOL (mmol/L) in human serum or plasma.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{C. Esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{C. Oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4H_2O$$

Cholesterols are hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. The cholesterol is oxidased by oxygen with the catalysis of cholesterol oxidase producing hydrogen peroxide. Hydrogen peroxide reacts with 4-aminoantipyrine in the presence of peroxidase to yield a red quinoneimine dye that shows an maximum absorbences at 520 nm.

The iecrease in absorbance at 520 nm is directly proportional to the CHOL concentration in the sample.

MATERIALS PROVIDED

Reagents:

R	Cholesterol esterase	≧1500 U/L
	Cholesterol oxidase	≧400 U/L
	Peroxidase	≧3000 U/L
	4-aminoantipyrine	2.0 mmol/L
	Phospate buffer, pH 6.8	1.8 mmol/L
	3,5 DHBS	50 mmol/L
	Sodium azide	1 g/L

MATERIALS NEEDED BUT NOT PROVIDED

1. Automated chemistry analyzer
2. CHOL calibrator set (commercially available)

INSTRUMENT

The Kit is applicable on most automated chemistry analyzers. Refer to specific instrument application for suggested settings.

STORAGE AND STABILITY

Store the reagents at 2-8℃. Avoid direct sunlight. The Kit is stable through the expiration date when stored properly. The reagent is stable for 1 month at 2-8℃ after opening.

PRECAUTIONS

1. The Kit is for *in vitro* diagnostic use only. Not for use in humans or animals.
2. The instructions must be followed to obtain accurate results.
3. Do not use the reagents beyond the expiration date.
4. Treat all specimens as infectious. Proper handling and disposal procedures of specimens and test materials should be strictly followed.

SPECIMEN COLLECTION AND HANDLING

Follow standard laboratory procedures to collect blood or serum preventing hemolysis.
It is recommended to perform test immediately after sample collection.

TEST PROCEDURE (see Figure 1)

Reagent is liquid stable ready-to-use, no preparation needed.

Calibration: Recommend using commercially available calibrator set for optimal results.

Test procedure: see Figure 1 and Table 1 for instrument parameter setup. Refer to specific instrument application for suggested setting.

1. Add 3 µl of sample and 300 µl of R1; mix well and incubate at 37℃ for 10 minutes.
2. Take optical density measurement.
3. Calculate average Δ A.

RESULT

The amount of CHOL in mmol/L can be obtained the following calculation:

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CHOL (mmol/L) = $\frac{\Delta A test}{\Delta A standard}$ × standard solution (mmol/L)

Please refer to instrument application if testing under different conditions.

EXPECTED VALUES

<5.2 mmol/L
It is recommended for each laboratory to establish its own expected values

QUALITY CONTROL

Using included Bioway controls with known concentration is recommended before each batch of tests to ensure the test is properly performed and all reagents and the instrument are functional as specified.

LIMITATIONS

1. The Kit is for *in vitro* use on automated chemistry analyzers only.
2. The test result from the Kit should not be used as the only basis for definite diagnosis.
3. Samples with CHOL exceeding the maximum measurement range should be diluted with saline and retested.

PERFORMANCE CHARACTERISTICS

Linearity: 0 – 17.5 mmol/L (R≥0.990)

Accuracy: Bias proportion 90%~110%

Precision: Within Run: CV≤3%;
Run-to-Run: CV≤5%

Interference: no interference detected for: Unconjugated bilirubin (≦684μmol/L), Ascorbic acid (≦50mg/dl), Bilirubin (≦684μmol /dl), Hemoglobin (≦ 500mg/dl), Heparin, EDTA and Sodium Fluoride in a normal dose.

Reagent Blank Absorbance: at 520nm wavelength and 10 mm optical diameter, O.D. ≤ 0.08.

REFERENCES

1. Allain C. C. *et al.*, Clin. Chem., 20:470 (1974.
2. Trinder P., Ann. Clin. Biochem., 6:24 (1969)
3. Witte D. L. *et al.*, Clin. Chem., 20:1282 (1974)
4. Perlstein M. T. *et al.*, J. Microchem., 22:403 (1977)

Not Intended for Sale in the United States.

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