Bioway Chemistry Reagent Series
The Serum HDL-C Reagent Kit

Detection of HDL-Cholesterol in Human Serum or Plasma on Chemistry Analyzers

Cat. No. R034K11 ***HDL-Cholesterol Reagent Kit***

SUMMARY OF TEST PROCEDURE

Figure 1: Procedure Diagram

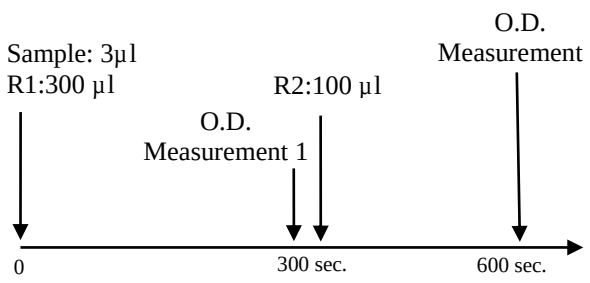

Table 1: Instrument Parameters*

Calibration method	linear	Slope of reaction	increase
Wavelength	600nm(prim.) 800nm (2nd)	Sample volume	3 µl
Test method	2 point end	R1 volume	300 µl
Reaction temperature	37℃	R2 volume	100 µl

*Refer to Figure 1 and the package insert for detail

INTENDED USE

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

SUMMARY AND EXPLANATION

Lipoproteins are spherical particles that transport cholesterol and triglycerides in the bloodstream. The different classes of lipoproteins are categorized by their densities which include: very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL), and chylomicrons (CM). HDL-cholesterol is known as "good" cholesterol because of HDL's cardio protective mechanism that uptakes and transports the cholesterol from peripheral tissue to the liver. Low levels of HDL-cholesterol have been reported to be associated with increased risk of coronary heart disease (CHD) and coronary artery disease (CAD). Measurement of HDL-cholesterol, in addition to measuring total cholesterol, is a useful way to identify high-risk patients for prevention and management of cardio related diseases.

TEST PRINCIPLES

The Kit utilizes a two-part, liquid stable reagent method for directly measuring HDL-C levels in human serum or plasma. The first reaction involves the elimination of all non-HDL lipoproteins through selective reaction with cholesterol esterase and cholesterol oxidase that is coupled to a colorless endpoint via catalase reduction of the peroxide byproduct. The catalase is then inhibited by sodium azide while the HDL specific surfactant solubilizes the HDL particles to release the HDL-cholesterol that then reacts with cholesterol esterase and cholesterol oxidase. In the presence of peroxidase the peroxide byproduct now reacts with 4-AAP and DSBmT to form a colored substance that is measured spectrophotometically at 600 nm, which is proportional to the amount of HDL-cholesterol in the sample.

MATERIALS PROVIDED

Reagents:

R1	DSBmT 0.25 mmol/L; Surfactant (Tween-80) 1.0g/L
R2	Cholesterol esterase 100 U/L; Cholesterol oxidase 6600 U/L; Peroxidase 2500 U/L;
R2	4-AAP 2.5 mmol/L Sodium azide 0.1g/L

*DSBmT: N,N-bis (4-sulfhobutyl)-m-Toluidine-disodium
4-AAP: 4-aminoantipyrine ascorbic acid oxidase

MATERIALS NEEDED BUT NOT PROVIDED

1. Sample collection tube for applicable instrument
2. HDL-C calibrator set and control set (available for purchase)

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. R1 and R2 reagents are 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

The Test can be performed with human serum, EDTA-treated or heparinized plasma. Follow standard laboratory procedures to collect specimens. In not analyzed immediately, specimens may be stored at 2-8 ℃ for up to 1 week or frozen at -20℃ for up to 1 month. For storage longer than 1 month, the sample should be kept at -70 ℃ for up to 2 years.

TEST PROCEDURE (see Figure 1)

Reagent 1 and 2 are liquid stable ready-to-use, no preparation needed.

Calibration: Recommend using Bioway calibrator set for optimal results. Please refer to Bioway HDL calibrator instruction for more detail.

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 300 seconds.
2. Take optical density measurement OD 1 at 300 seconds.
3. Add 100 µl of R2, mix well and incubate at 37℃.
4. Take optical density measurement OD 2 at 600 seconds.
5. Absorbance= OD2-OD1.

RESULT

The HDL-cholesterol concentration in mmol/L can be obtained by

comparing the absorbance to the corresponding value on the calibration curve.

EXPECTED VALUES

Male: 0.78 – 1.81 mmol/L

Female: 0.78 – 2.20 mmol/L

National Cholesterol Education Program guidelines for CHD risk factor:

<0.90 mmol/L	low HDL-C (major risk factor)
>1.55 mmol/L	high HDL-C (negative risk factor)

It is recommended for each laboratory to establish its own expected values

QUALITY CONTROL

Commercially available HDL controls may be used. It is recommended to use Bioway HDL Control set (available for purchase) for optimal results.

A control should be tested before each batch of tests to ensure the test is properly performed and all reagents and the instrument are functional as specified. The recovery of control values should be within the appropriate range. Please see Bioway HDL Control set package insert for more detail.

LIMITATIONS

1. The Kit is for *in vitro* use on automated chemistry analyzers only.
2. Anticoagulants containing citrate should not be used.
3. Results obtained using the Kit should not be used as the only basis for a definite diagnosis.
4. Samples with values greater than 3.9 mmol/L should be diluted 1:1 with saline and re-tested.
5. Consumption of large doses of vitamin C could cause false lowering of the HDL-C value.
6. Hemolysis sample will cause inaccurate results.
7. For samples with Triglyceride greater than 9.04 mmol/L (800 mg/dL) or total cholesterol greater than 12.9 mmol/L (500 mg/dL), the sample should be diluted with saline and re-tested.

PERFORMANCE CHARACTERISTICS

Linearity: 0- 3.9 mmol/L (R≥0.990)

Accuracy: control recovery relative deviation ≤10%;

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

Interference: no interference detected for: Bilirubin (20 mg/dL), ascorbic acid (40 mg/dL), and hemoglobin (450 mg/dL)

Reagent Blank Absorbance: at 600nm wavelength and 10 mm optical diameter, O.D. ≤ 0.10

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Not Intended for Sale in the United States.

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