Bioway Chemistry Reagent Series

The Serum LDL-C Reagent Kit

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

Cat. No. R042K11 ***LDL-C Reagent Kit***

SUMMARY OF TEST PROCEDURE

Figure 1: Procedure Diagram

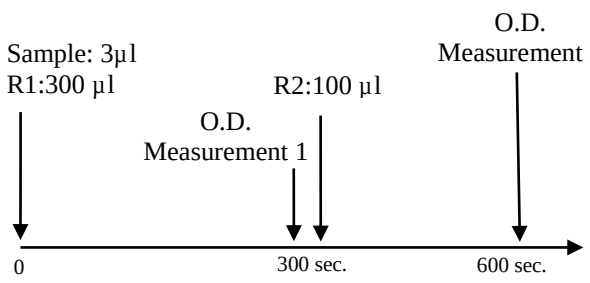

Table 1: Instrument Parameters*

Calibration method	linear	Slope of reaction	increase
Wavelength	546nm(prim.) 660nm (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 **LDL-Cholesterol Reagent Kit** (the Kit) is an assay intended for *in vitro* quantitative detection of Low Density Lipoprotein-Cholesterol (LDL-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). LDL-cholesterol is known as "bad" cholesterol, because studies have shown that elevated levels of LDL-cholesterol is an important risk factor for cardiovascular diseases, particularly in causing artherosclerosis and coronary artery disease (CAD). It is important to test for LDL-cholesterol in addition to total cholesterol because even with normal total cholesterol level, an increase in LDL-cholesterol can occur with increased risk for CAD.

TEST PRINCIPLES

The Kit utilizes a two-part, liquid stable reagent method for directly measuring LDL-C levels in human serum or plasma. Reagent 1 contains polyanions that form compound with LDL, while the surfactant solubilizes all other non-LDL particles. In the absence of a coupler, the cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a colorless reaction. Reagent 2 contains LDL specific surfactant that solubilizes the LDL particles to release the LDL-cholesterol that reacts with cholesterol esterase and cholesterol oxidase. In the presence of peroxidase the peroxide byproduct now reacts with 4-AAP and TOOS to form a colored substance that is measured spectrophotometically at 546 nm, which is proportional to the amount of LDL-cholesterol in the sample.

MATERIALS PROVIDED

Reagents:

R1	TOOS 0.96 mmol/L; Polyanion 2.0 mmol/L; Surfactant (Tween-80) 1.0g/L
R2	Cholesterol esterase 100 U/L; Cholesterol oxidase 6600 U/L; Peroxidase 2500 U/L; 4-aminoantipyrine ascorbic acid oxidase 2.5 mmol/L

MATERIALS NEEDED BUT NOT PROVIDED

1. Sample collection tube for applicable instrument
2. LDL-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 5 days or frozen at -80℃ for up to 2 weeks.

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 LDL 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 LDL-cholesterol concentration in mmol/L can be obtained by comparing the absorbance to the corresponding value on the calibration curve.

EXPECTED VALUES

The following are the National Cholesterol Education Program suggested values for prevention and management of coronary heart

disease:

LDL Cholesterol

Desirable:	<3.36 mmol/L
Borderline High Risk:	3.36 – 4.11 mmol/L
High Risk	>4.14 mmol/L

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

QUALITY CONTROL

Commercially available LDL controls may be used. It is recommended to use Bioway LDL 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 LDL 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 11.7 mmol/L should be diluted 1:1 with saline and re-tested.

PERFORMANCE CHARACTERISTICS

Linearity: 0- 11.7 mmol/L (R≥0.995)

Accuracy: 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), hemoglobin (450 mg/dL), sodium citrate (1000 mg/dL), EDTA Na2 (200 mg/dL), and heparin (5000 U/dL)

Reagent Blank Absorbance: at 546nm wavelength and 10 mm optical diameter, O.D. ≤ 0.10

REFERENCES

1. Badimon, J.J., Badimon L., Fuester V., Regression of Athroscierotic Lesions by High-density lipoprotein Plasma fraction in the Cholesterol-Fed Rabbit, Journal of Clinical Investigation, 85:1234-41 (1990).
2. Barr, D.P., Russ, E.M, Elder, H.A., Protein-Lipid Relationships in Human Plasma, Am. J. Med. 11:480 (1951).
3. Castelli, W.P., et al., Cholesterol and other Lipids in coronary heart disease, Circulation, 55-767 (1977).
4. Crouse, J.R., et al., Studies of Low Density Lipoprotein Molecular Weight in Human Beings with Coronary Artery Disease, J. Lipid Res., 26:566 (1985).
5. Gordon, T., et al, High Density Lipoprotein as a Protective Factor Against Coronary Heart Disease, Am. J. Med., 62:707 (1977).
6. Gotto, A.M., Lipoprotein Metabolism and the etiology of Hyperlipidemia,Hospital practice, 23:Suppl. 1,4 (1988).
7. Kannel, W.B., Castelli W.P., Gordon, T., Cholesterol in the Prediction of Artheriosclerotic Disease; New Perspectives Based on the Framingham Study, Am. Intern. Med., 90:85 (1979).
8. William, P., Robinson, D., Baily A., High Density Lipoprotein and Coronary Risk Factor, Lancet, 1:72 (1979).

Not Intended for Sale in the United States.

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