

LDL (Auto-Easy) Reagent

Catalog #: 43726

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

This reagent is intended for the *in vitro* quantitative determination of low-density lipoprotein cholesterol (LDL-C) in human serum.

SUMMARY AND EXPLANATION

Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipids, free cholesterol and protein constitute the outer surface of the lipoprotein particle, while the inner core contains mostly esterified cholesterol and triglyceride. These particles serve to solubilize and transport cholesterol and triglyceride in the bloodstream. The relative proportions of protein and lipid determine the density of these lipoproteins and provide a basis on which to begin their classification. These classes are: chylomicrons, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have very distinct and varied effects on coronary heart disease risk. The studies all point to LDL cholesterol as the key factor in the pathogenesis of atherosclerosis and coronary artery disease (CAD), while HDL cholesterol has been observed to have a protective effect. Even within the normal range of total cholesterol concentrations, an increase in LDL cholesterol can occur with an associated increased risk for CAD.

METHODOLOGY

SDI LDL Cholesterol Auto-Easy Reagent is an enzyme selective reagent method. The method is in a two reagent format. When a sample is mixed with Reagent 1, the protecting reagent binds to LDL, and protects LDL from enzyme reactions. Cholesterol esterase and cholesterol oxidase react with non-LDL lipoprotein, very low density lipoprotein (VLDL) and HDL. Hydrogen peroxide produced by the enzyme reactions with non-LDL reactions with non-LDL cholesterol is decomposed by catalase in Reagent 1. When Reagent 2 is added, the protecting reagent is removed from LDL and catalase is inactivated by sodium azide. In this second process, cholesterol esterase and cholesterol oxidase react only with LDL-C. Hydrogen peroxide produced by the enzyme reactions with LDL-C yields a color complex upon oxidase condensation with N-(2-hydroxy-3-sulfopropyl)-3-5-dimethoxyaniline and 4-aminoantipyrine in the presence of peroxidase. By measuring the absorbance of the blue color complex product, at approximately 600nm, the LDL-C concentration in the sample can be calculated when compared with the absorbance of the LDL-C Calibrator.

REAGENT COMPOSITION

Active Ingredients	Concentration
Reagent 1	
Good's Buffer	25 mM
Cholesterol esterase (Pseudomonas)	5,000 U/L
Cholesterol oxidase (Nocardia)	5,000 U/L
N-(2-hydroxy-3-sulfopropyl)-3-5-dimethoxyaniline and 4-aminoantipyrine	0.8 mM
Anti human <i>b</i> -lipoprotein antibody	0.64 mM
Catalase (Bovine liver)	1,000,000 U/L
pH 6.8 ± 0.1	
Reagent 2	
Good's Buffer	25 mM
4-aminoantipyrine	3.4 mM
Peroxidase (Horseradish)	20,000 U/L
NaN ₃	0.1 %
pH 7.0 ± 0.1	

Precautions and Warnings:

- For in vitro diagnostic use only.
- DO NOT pipette by mouth. Avoid contact with skin and eyes.
- If spilt, thoroughly, wash affected areas with water. For further information, consult the SDI LDL Enzyme Selective Reagent Material Safety Data Sheet.
- All specimens used in the test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing.
- Do not use the reagent after the expiration date printed on the kit.

REAGENT PREPARATION

Reagent 1: Supplied ready to use.
Reagent 2: Supplied ready to use.

REAGENT STORAGE

- Store the reagent at 2-8°C.
- The reagent is stable until the expiration date printed on the label when stored at 2-8°C.

REAGENT DETERIORATION:

DO NOT USE REAGENT IF:

- The reagent develops turbidity.
- The reagent fails to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING

Sample: Serum is the recommended specimen.

Storage: If not analyzed promptly, specimens may be stored at 2-8°C for up to 5 days. If specimens need to be stored for longer than 5 days, they may be stored frozen at -80°C.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- SDI CA480 Clinical Chemistry System.
- Analyzer specific consumables, eg: sample cups.
- LDL Control and LDL Calibrator materials such as those provided by SDI Biomed.

ASSAY PROCEDURE

LDL Cholesterol (Enzyme Selective)

Temperature	37°C
Primary Wavelength	600 nm
Secondary Wavelength	700 nm
Assay Type	Endpoint
Direction	Increase
Sample Vol.	3 mL
Reagent 1 Vol.	270 mL
Incubation Time	5 minutes
Reagent 2 Vol.	90 mL
Incubation Time	5 minutes

Calculations

$$\frac{A(\text{patient})}{A(\text{standard})} \times \text{Concentration of standard (mg/dL)} = \text{LDL Cholesterol (mg/dL)}$$

Limitations:

- Unit Conversion: mg/dL x 0.02586 = mmol/L
- Protect reagents from direct sunlight and store as directed.
- Young, D.S. has published a comprehensive list of drugs and substances which may interfere with this assay.

CALIBRATION

The assay requires the use of SDI HDL/LDL Cholesterol- Calibrator. The value of the SDI LDL Cholesterol—Calibrator is assigned by procedures traceable to NIST SRM 1951a.

Refer to appropriate instrument operator manual for calibration interval.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known LDL Cholesterol values. SDI HDL/LDL Cholesterol Control Kit - Levels 1 & 2 are recommended.

The National Cholesterol Education Program (NCEP) in the USA has recommended that two levels of controls, one in the normal range (<100 mg/dL) and one in the high risk range (>160 mg/dL), be run in the same manner as patient samples. An acceptable range of LDL Cholesterol values should be established with this method by repeat analysis. Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.

INTERFERENCES

Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out according to NCCLS No EP7-P. The following results were obtained:

Hemoglobin:

No significant interference from hemoglobin up to 500 mg/dL.

Bilirubin:

No significant interference from bilirubin up to 50 mg/dL.

A number of drugs and substances may affect the accuracy of this test. See Young, et al.¹²

EXPECTED VALUES

The following NCEP cutpoints for patient classification are used for the prevention and management of coronary heart disease.

LDL Cholesterol	Classification
<100 mg/dL	Optimal
100-129 mg/dL	Near optimal /above optimal
130 – 159 mg/dL	Borderline high
160 – 189 mg/dL	High
≥190 mg/dL	Very High

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE

Linearity:

When run as recommended the assay is linear from 1 to 400 mg/dL

Method Comparison:

Accuracy or serum correlation studies on the SDI LDL Cholesterol Enzyme Selective reagent method was performed by comparisons to another commercially available direct LDL Method.

Number of samples pairs:	60
Correlation Coefficient:	0.986
Slope:	1.018
Intercept (mg/dL):	0.1

Precision:

Within Run	Level 1	Level 2
n=10		
Mean (mg/dL)	101.2	164.5
S.D. (mg/dL)	0.6	0.7
C.V. (%)	0.6	0.4
Total		
n=80 (20 days / 2 runs per day / 2 replicates per run)		
Mean (mg/dL)	126.2	225.8
S.D. (mg/dL)	0.8	1.2
C.V. (%)	0.6	0.5

REFERENCES

- Gotto A.M., Lipoprotein metabolism and the etiology of hyperlipidemia, Hospital Practice, 23:Suppl.1, 4 (1988).
- 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).
- Badimon J.J., Badimon L., Fuster V., Regression of Atherosclerotic Lesions by High-Density Lipoprotein Plasma Fraction in the Cholesterol-Fed Rabbit, Journal of Clinical Investigation, 1990; 85:1234-41.
- Castelli W.P. et al., Cholesterol and other lipids in coronary heart disease, Circulation, 55:767 (1977).
- Barr D.P., Russ E.M., Eder H.A., Protein-lipid relationships in human plasma, Am. J. Med., 11:480 (1951).
- Gordon T. et al., High density lipoprotein as a protective factor against coronary heart disease, Am. J. Med., 62:707 (1977).
- William P., Robinson D., Baily A., High density lipoprotein and coronary risk factor, Lancet, 1:72 (1979).
- Kannel W.B., Castelli W.P., Gordon T., Cholesterol In the prediction of atherosclerotic disease; New perspectives based on the Framingham study, Am. Intern. Med., 90:85 (1979).
- Beharok P.S. et al., National Cholesterol Education Program Recommendations for Measurement of Low-Density Lipoprotein Cholesterol; Executive Summary, Clinical Chemistry, Vol. 41, No. 10, 1995.
- Grundy S.M. et al., Summary of the Second Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II), JAMA 1993, 269:23:3015-3023.
- National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation Protocol Number 7, Vol.4, No.8, June 1984.
- Young D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., AACCC Press, Washington DC, 1990, 3:104 thru 3:106.
- Tietz N.W., Clinical Guide to Laboratory Tests, W.B. Saunders Co., Philadelphia, 1986, p. 256.
- National Institutes of Health Publication No. 01-3670, May, 2001.

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