

This package insert must be read carefully prior to use.

LDL-cholesterol assay kit  
(Classification No.: 30173000)

## QUALIGENT LDL

### General Precautions

1. This product is for in vitro diagnostic use, and must not be used for any other purposes.
2. Clinicians should make a comprehensive clinical decision based on assay results in conjunction with clinical symptoms and other examination results.
3. This product should be used only as directed in this package insert. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.
4. If the reagent accidentally comes in contact with eyes and/or mouth, rinse immediately with ample water as first aid, and consult the doctor if required.
5. Carefully read the operating instructions for each type of automated analyzers prior to using this product. Parameters for each type of analyzers are available, and can be requested from SEKISUI MEDICAL CO., LTD. if required.
6. Perform a quality control test prior to assay to ensure accuracy.

### Description (Kit Components) \*\*

Component : Ingredients

LDL Enzyme Solution 1:

4-Aminoantipyrine  
Cholesterol oxidase (microbial origin)  
Cholesterol esterase  
Peroxidase  
Surfactant 1  
Good's buffer

LDL Coloring Solution 2:

N,N-bis  
(4-sulfobutyl)-m-toluidine disodium (DSBmT)  
Surfactant 2  
Good's buffer

### Intended Use

#### Measurement of LDL-cholesterol in serum or plasma

Cholesterol that exists in LDL (Low Density Lipoprotein) fraction is called LDL-cholesterol. Elevation of LDL-cholesterol is known to be a risk factor for arteriosclerotic diseases, especially coronary artery disease.

Total cholesterol has commonly been measured for diagnosis of hyperlipidemia, which is considered to be a cause of arteriosclerotic diseases. However, according to the "Research Report 1986 on Specified Disease Primary Hyperlipidemia by Investigation and Research Group of the Ministry of Health and Welfare"<sup>1)</sup>, the LDL-cholesterol level is more closely

correlated with ischemic heart disease than the total cholesterol level.

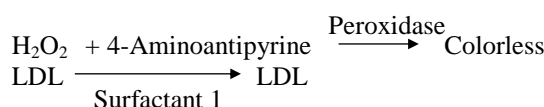
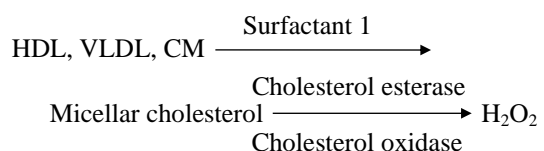
The LDL-cholesterol concentration has been calculated according to the Friedwald equation based on measurement of 3 other variables (total cholesterol, HDL-cholesterol, and triglycerides). However, the accuracy of this method is insufficient. Even though ultracentrifugation has been also performed as the standard method of measuring LDL-cholesterol, this method is difficult to use for laboratory tests in routine clinical practice as requires special equipment and time. The direct method, therefore, is widely employed in recent years.

### Assay Principle

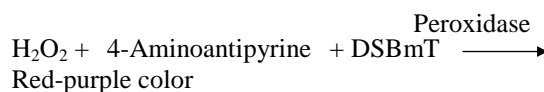
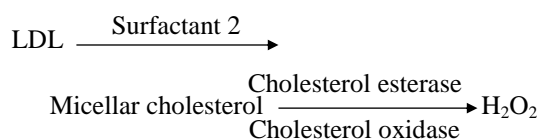
#### 1. Assay Principle

The principle of this method is that each lipoprotein reacts differently with surfactants depending on its physicochemical properties. Therefore, two different surfactants are employed. Surfactant 1 is added in the first reaction. It can change the structure of lipoproteins other than LDL, including chylomicrons (CM), VLDL and HDL. In the presence of this surfactant, lipoproteins other than LDL are eliminated by the action of cholesterol oxidase and cholesterol esterase. Surfactant 2, which promotes the enzymatic reaction of all types of lipoproteins, is used in the second reaction. The enzymatic reaction with LDL-cholesterol left from the first reaction is initiated to produce color development.

First reaction:



Second reaction:



#### 2. Features

- 1) The direct method does not require complicated procedures.
- 2) Results obtained by this method are closely correlated with those by ultracentrifugation.
- 3) Analytical results are not affected by chyle (up to TG 1500 mg/dL).

## Procedural Precautions \* \*

### 1. Properties of Samples and Sampling Methods

#### 1) Samples

Serum and plasma (heparin plasma and EDTA plasma) may be used.

#### 2) Storage of samples<sup>5)</sup>

If the isolated serum or plasma sample cannot be tested on the same day, specimens should be stored as follows:

2–10°C: for tests within 1 week

≤ -20°C: for tests after more than 1 week

Do not re-freeze.

Bring samples to room temperature

(15–30°C) before use.

### 2. Interfering substances

Assay results are not affected by free bilirubin (up to 20 mg/dL), conjugated bilirubin (up to 20 mg/dL), hemoglobin (up to 500 mg/dL), ascorbic acid (up to 50 mg/dL), formazin turbidity (up to 3000 FTU), or Intralipos (up to 5%).

### 3. Others

1) Always use Cholestest N Calibrator for calibration.

However, use QUALIGENT N Calibrator for LABOSPECT 008 (manufactured by SEKISUI MEDICAL) as the calibration material with a Hitachi LABOSPECT 008 Automated Analyzer.

2) Precautions for assay range

If the concentration of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

## Dosage/Administration (Assay Procedure) \* \*

### 1. Preparation of reagents

Reagent (1): LDL Enzyme Solution 1 is ready to use.

Reagent (2): LDL Coloring Solution 2 is ready to use.

### 2. Assay Procedure

This product is compatible with Hitachi 9000 series and LABOSPECT series automated analyzers. Assay procedure is indicated below.

Sample	+	Reagent (1)	$\xrightarrow[5 \text{ min}]{37^\circ\text{C}}$	Measurement
2 $\mu\text{L}$		180 $\mu\text{L}$		(Absorbance I <sup>*</sup> )
		Reagent (2)	$\xrightarrow[5 \text{ min}]{37^\circ\text{C}}$	Measurement
		60 $\mu\text{L}$		(Absorbance II <sup>*</sup> )
			$\longrightarrow$	Calculation of concentration

\*Absorbance I and II: The difference in absorbance between 660 nm and 546 nm.

Calibration material: Cholestest N Calibrator

Use QUALIGENT N Calibrator for LABOSPECT 008 (manufactured by SEKISUI MEDICAL) as the calibration material with a Hitachi LABOSPECT 008 Automated Analyzer. (Manufacture's assigned value)

Reagent blank: Purified water or saline

## Assessment of Assay Results \* \*

### 1. Reference standard range<sup>3)</sup>

60–163 mg/dL

### 2. Diagnostic criterion<sup>4)</sup>

Hyper-LDL cholesterolemia:  $\geq 140$  mg/dL

3. There may be reactions with non-target substances in the samples or interfering reactions. Especially if abnormal lipid metabolism is suspected including cases of biliary obstruction, accurate measurements may not be obtained due to the influence of lipoprotein abnormalities. If assay results appear to be unreliable, repeat the measurement (if necessary, after dilution) or try another analytical methods.

## Performance \* \*

### 1. Sensitivity

1) Reagent blank: absorbance being equal to or lower than 0.05

2) Sensitivity :The absorbance is 0.18–0.28 per 100 mg/dL of LDL-cholesterol.

2. Accuracy: 90–110% of the expected assay value

### 3. Within-run Reproducibility:

Coefficient of variation  $\leq 5\%$

(Test methods used for 1. –3. are in-house methods.)

4. Measurement Range<sup>5)</sup>: (On a Hitachi 9000 series automated analyzer)

1–400 mg/dL

### 5. Correlation<sup>5)</sup>

1) Serum N=70  $r=0.997$   $y=0.96x+0.02$

Control method: Approved in vitro diagnostic (enzymatic assay)

2) Plasma N=60  $r=0.999$   $y=1.03x-1.14$

Control method: Approved in vitro diagnostic (enzymatic assay)

### 6. Standard Material

Certified Reference Material for Measurement of HDL Cholesterol, LDL Cholesterol and Triglycerides in Human Serum (Reference Material Institute for Clinical Chemistry Standards)

## Precautions for Use or Handling \* \*

### 1. Precautions for Handling (to Ensure Safety)

1) All samples used in the test should be handled as a material possibly infected with HIV, HBV, HCV, or other viruses. To prevent infection, use disposable gloves and avoid mouth pipetting during the test.

2) Cholestest N Calibrator and QUALIGENT N Calibrator for LABOSPECT 008 contain human-derived components that have been shown to be negative for HBs antigens, HIV antibodies (AIDS virus antibodies), and HCV antibodies. However, these reagents (as well as the samples) should be considered potentially infectious and handled with great care..

3) Proclin 300, which possesses skin-irritative potential, is added as an antiseptic agent in the LDL Enzyme Solution 1 and LDL Coloring Solution 2. Therefore, if the reagent comes in contact with skin or clothes, rinse immediately with ample water, and consult the doctor if skin irritation develops.

### 2. Precautions for use

1) This product should be stored as directed, without freezing. Freezing can deteriorate the

reagents, which can produce inaccurate results. Therefore, avoid using the reagents which have been previously frozen.

- 2) Do not use expired reagents. Use of such reagents cannot guarantee the reliability of measurement values.
- 3) Do not perform the assay under direct sunlight

### 3. Precautions for Disposal

- 1) Before disposal, used samples and their containers must be immersed in sodium hypochlorite solution at a concentration of greater than 0.1% for longer than 1 hour or autoclaved at 121°C for 20 minutes.
- 2) To prevent infections from spilled samples or solutions containing samples, wipe the spilled area thoroughly with disinfectants such as sodium hypochlorite solution at a concentration of greater than 0.1%.
- 3) The reagents and treated samples should be discarded as medical waste or industrial waste according to the waste disposal regulations.
- 4) The reagents should be disposed of in accordance with the Water Pollution Control act

### 4. Other precautions

- 1) Do not use the containers for other purposes.
- 2) Do not take apart the reagent cartridge before.

### Storage and Shelf Life \*\*

1. Storage temperature: 2–10°C
2. Shelf life: 2 years from the date of manufacture (The expiration date is printed on the outer package.)

### Packaging \* \*

Name	Package		
QUALIGENT LDL	Set (Cassette for Hitachi 9000 series)	LDL Enzyme Solution 1 1 × 27.0 mL	× 2
		LDL Coloring Solution 2 1 × 9.0 mL	
	L set (Set for Hitachi LABOSPECT series)	LDL Enzyme Solution 1 1 × 48 mL	× 2
		LDL Coloring Solution 2 1 × 16 mL	

### References \*\*

- 1) Tarui S.: Research Report 1986 on Specified Disease Primary Hyperlipidemia by Investigation and Research Group of the Ministry of Health and Welfare (group leader: Seiichi Tarui), 17–26, 1987.
- 2) Kanno T. et al.: J Med Pharm Sci, 37, 635–644, 1997.
- 3) Kanai M. (supervising editor): Kanai's manual of clinical laboratory medicine. 34th ed. 522, Kanehara Shuppan, 2015.
- 4) Japan Atherosclerosis Society, ed. 2012 Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases, 13.
- 5) In house data, SEKISUI MEDICAL CO., LTD.

### Contact

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### Manufacturer \*\*

**SEKISUI MEDICAL CO., LTD.**

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