This package insert follows the Pharmaceuticals, Medical devices and Other Therapeutic Products Act of Japan.

In Vitro Diagnostics
Marketing Notification No.13A2X00197218042

**Revised: January 2017 (13th edition)
*Revised: September 2016 (12th edition)

This package insert must be read carefully prior to use.

HDL-cholesterol assay kit
(Classification No.: 30169000)

Cholestest N HDL

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

Enzyme Solution 1:	N,N-bis (4-sulfobutyl)-m-toluidine disodium (DSBmT) Cholesterol oxidase (microbial origin) Peroxidase
Enzyme Solution 2:	4-Aminoantipyrine Cholesterol esterase Surfactant

Intended Use

Measurement of HDL-cholesterol in serum or plasma

Cholesterol that exists in HDL (High Density Lipoprotein) fraction is called HDL-cholesterol. The Framingham study (1977) and other studies have shown that a low level of HDL-cholesterol is a risk factor for ischemic heart disease.[1]
HDL-cholesterol is decreased in patients with coronary artery disease, hyperlipidemia, smoking, obesity, diabetes, and hepatic dysfunction, while it is increased by alcohol intake and by adequate exercise. The HDL-cholesterol concentration also varies depending on age, gender, and hereditary factors.

Assay Principle

1. Assay Principle

In this assay system, HDL-cholesterol is selectively solubilized, and rapidly undergoes an enzymatic reaction due to the use of special surfactant with different effects on HDL and other lipoproteins (LDL, VLDL and chylomicron). Therefore, only HDL-cholesterol is specifically measured.

$$\text{HDL, LDL, VLDL, Chylomicron} \xrightarrow{\text{Surfactant}} \text{HDL is solubilized}$$

$$\text{HDL-cholesterol} \xrightarrow{\text{Cholesterol esterase}} \Delta^4\text{-cholestenone} + H_2O_2$$

$$H_2O_2 + \text{DSBmT} + \text{4-Aminoantipyrine} \xrightarrow[\text{Cholesterol oxidase}]{\text{Peroxidase}} \text{Red-purple color}$$

2. Features

1) This product is used to measure HDL-cholesterol by the direct method.
2) Because it does not contain Mg salt, there is no heavy burden imposed on analyzer.

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[3)4)]
If a serum or plasma sample cannot be measured on the day of separation, store the sample 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 50 mg/dL), conjugated bilirubin (up to 50 mg/dL), hemoglobin (up to 500 mg/dL), ascorbic acid (up to 50 mg/dL), or Intralipos (up to 5%).

3. Others

1) Always use Cholestest N Calibrator or QUALIGENT N Calibrator for calibration.
2) Precautions for assay range
If the concentration of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

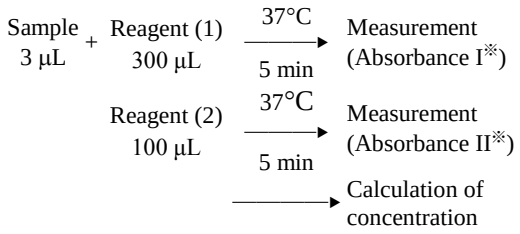
Dosage/Administration (Assay Procedure)

1. Preparation of reagents

Reagent (1): Enzyme Solution 1 is ready to use.
Reagent (2): Enzyme Solution 2 is ready to use.

2. Assay Procedure

This product is compatible with various types of automated analyzer. An example of the assay procedure is indicated below.



※Absorbance I and II: The difference in absorbance between 700 nm and 600 nm.
Calibration material: Cholestest N Calibrator (in-house indicated values) or QUALIGENT N Calibrator for LABOSPECT 008 (Manufacture's assigned value)
Reagent blank: Purified water or saline

Assessment of Assay Results **

1. **Reference standard range**[1)]
   Male: 38–90 mg/dL
   Female: 48–103 mg/dL
2. **Diagnostic criterion**[2)]
   Low-HDL cholesterolemia: < 40 mg/dL
3. There may be reactions with non-target substances in the samples or interfering reactions. 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: 0.07–0.17 per 100 mg/dL of HDL-cholesterol.
2. **Accuracy**: 90–110% of the expected assay value
3. **Within-run Reproducibility:**
   Coefficient of variation ≤ 5 %
   (Test methods used for 1. –3. are in-house methods.)
4. **Measurement Range**[4)]: (On Hitachi 7170 automated analyzer)
   2–150 mg/dL
5. **Correlation**[4)]
   1) Serum N=56 r=0.999 y=0.99x+0.39
      Control method: Approved in vitro diagnostic (selective inhibition assay)
   2) Plasma N=76 r=0.996 y=0.97x +0.28
      Comparison with the values for plasma samples obtained simultaneously with the serum samples.
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 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 Enzyme Solution 1 and Enzyme 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 replenish the reagents.
4) 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 121ºC 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 or related regulations.

4. Other precautions

Do not use the containers for other purposes.

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
Cholestest N HDL	Enzyme Solution 1	2 × 60 mL
	Enzyme Solution 2	2 × 20 mL

Constituent reagents are available in other configurations. For further details please contact SEKISUI MEDICAL CO., LTD.

References **

1) Kanai M. (supervising editor): Kanai's manual of clinical laboratory medicine. 34th ed. 520, Kanehara Shuppan, 2015.

2) Japan Atherosclerosis Society, ed. Japan Atherosclerosis Society (JAS) Guidelines for Diagnosis and Treatment of Atherosclerotic Cardiovascular Diseases, 2002, 5.
3) Kodajima N. et al.: J Med Pharm Sci, 46, 235, 2001.
4) In house data, SEKISUI MEDICAL CO., LTD.

Contact

SEKISUI MEDICAL CO., LTD.
international@sekisui.com

Manufacturer **

SEKISUI MEDICAL CO., LTD.
1-3, Nihonbashi 2-chome, Chuo-ku, Tokyo, Japan

This product uses a patent related to HDL-C measuring reagent and method (patent No. 3288033), and patent application (PCT/JP00/03860).