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

In Vitro Diagnostics
Marketing Notification No. 13A2X00197218047

**Revised: January 2017 (9th edition)
*Revised: April 2008 (8th edition)

This package insert must be read carefully prior to use.

Cholesterol assay kit
(Classification No.: 30159000)

Pureauto S CHO−N

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

CHO-N Enzyme Solution 1:
4-Aminoantipyrine
Cholesterol esterase
Peroxidase
Ascorbate oxidase
Phthalate buffer

CHO-N Enzyme Solution 2:
Cholesterol oxidase (microbial origin)
N-ethyl-N-sulfobutyl-m-toluidine sodium
Phthalate buffer

Intended Use

Measurement of total cholesterol in serum

Cholesterol in serum is derived from dietary intake and is also synthesized in the liver. Esterified and free cholesterol account for approximately 70% and 30% of total cholesterol, respectively.
Measurement of cholesterol is important as an index of abnormal lipid metabolism. The esterified/free cholesterol ratio is also considered to be an important index of severe parenchymal liver disease.

Assay Principle

1. **Assay Principle**
   Cholesterol exists in samples as both the esterified and free types. Esterified cholesterol is converted to free cholesterol by action of cholesterol esterase (CE). Then free cholesterol is oxidized by cholesterol oxidase (COD) to form hydrogen peroxide. Hydrogen peroxide causes oxidative condensation of 4-aminoantipyrine and N-ethyl-N-sulfobutyl-m-toluidine (ESBmT) in the presence of peroxidase (POD) to form a red-purple color. The total cholesterol content is determined by measuring the absorbance. The influence of ascorbic acid is blocked by ascorbate oxidase.

$$\text{Esterified cholesterol} \xrightarrow{\text{CE}} \text{Free cholesterol} + \text{Fatty acid}$$

$$\text{Free cholesterol} \xrightarrow{\text{COD}} \Delta^4\text{-cholestenone} + H_2O_2$$

$$H_2O_2 + \text{ESBmT} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red-purple color}$$

2. **Features**
   1) Liquid reagents, ready-to-use.
   2) Wide assay range.
   3) Assay results are hardly affected by cross contamination.
   4) Bilirubin, hemolysis, ascorbic acid and chyle have minimal effects on results.
   5) Applicable to various automated analyzers.

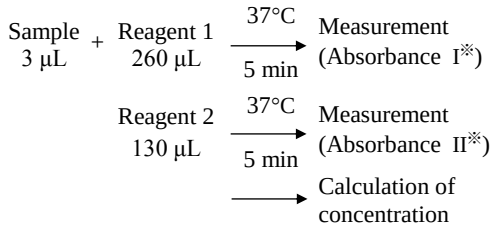
Procedural Precautions * *

1. **Properties of Samples and Sampling Methods**
   1) Samples
   Serum may be used.
   2) Storage of samples[1)]
   If the isolated serum 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
   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), and ascorbic acid (50 mg/dL).
3. **Others**
   1) Always use Seronorm Lipid or Cholestest 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): CHO-N Enzyme Solution 1 is ready to use.
   Reagent (2): CHO-N 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: Seronorm Lipid or Cholestest N Calibrator (Manufacture's assigned value)
Reagent blank: Purified water or saline

Assessment of Assay Results **

1. Reference standard range[2)]
142–248 mg/dL

2. Diagnostic criterion[3)]
Hypercholesterolemia: ≥ 220 mg/dL

3. There may be reactions or interfering reactions with non-target substances. 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 0.34–0.47 per 300 mg/dL of cholesterol.

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

3. Within-run Reproducibility:
Coefficient of variation ≤3%
(Test methods used for 1.–3. are in-house methods.)

4. Measurement Range[4)]: (On Hitachi 7170S automated analyzer)
4–1300 mg/dL

5. Correlation[4)]
1) Serum N=100 r=0.999 y=1.01x–3.85
Control method: Approved in vitro diagnostic (enzymatic method)

6. Standard Material
SRM911（NIST）

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) Sodium azide is added as an antiseptic agent in the CHO-N Enzyme Solution 2. Therefore, if the reagent comes in accidentally contact with eyes, mouth or skin, rinse immediately with ample water as first aid, and consult the doctor if required.
3) Proclin 300, which possesses skin-irritative potential, is added as an antiseptic agent in the CHO-N Enzyme Solution 1. 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.
5) Sodium azide has been added as an antiseptic agent in the CHO-N Enzyme Solution 2. It can react with lead or copper pipes to produce the highly explosive metal azide. Therefore, the reagent should be flushed with large amounts of water during disposal.

4. Other precautions
Do not use the containers for other purposes.

Storage and Shelf Life **

1. Storage temperature: 2–10°C

2. Shelf life: 18 months from the date of manufacture
(The expiration date is printed on the outer package.)

Packaging

Name			Package
Pureauto S CHO−N	(1)	CHO-N Enzyme Solution 1	2 × 400 mL
	(2)	CHO-N Enzyme Solution 2	2 × 200 mL

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

References * *

1) Sasaki M. et al.: Sampling of constituents of the human body, 246, Kodansha, 1972.
2) Kanai M. (supervising editor): Kanai's manual of clinical laboratory medicine. 34th ed. 512, Kanehara Shuppan, 2015.
3) Japan Atherosclerosis Society, ed. 2002 Japan

Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases, 5.
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