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

In Vitro Diagnostics
Marketing Notification No. 13A2X00197218049

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This package insert must be read carefully prior to use.

Triglyceride assay kit
(Classification No.: 30182000)

Cholestest TG

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:
Glycerol kinase (microbial origin)
Glycerol-3-phosphate oxidase (microbial origin)
N-Ethyl-N-sulfobutyl-*m*-toluidine sodium
Enzyme Solution 2:
4-Aminoantipyrine
Lipoprotein lipase
Peroxidase

Intended Use

Measurement of triglycerides in serum or plasma

Triglycerides are formed by esterification between 3 fatty acid molecules and 1 glycerol molecule, and are the major component of lipids throughout the body. Measurement of triglycerides is considered to be useful for elucidating abnormalities of lipid metabolism. Attention has especially been focused on the association of triglycerides with arteriosclerosis and coronary artery disease.

Assay Principle

1. Assay Principle

First reaction: Free glycerol in the sample is converted to glycerol-3-phosphate by glycerol kinase (GK) in the presence of adenosine triphosphate disodium (ATP). Then hydrogen peroxide is produced by glycerol-3-phosphate oxidase (GPO), after which hydrogen peroxide is decomposed into water and oxygen by catalase and these products are eliminated.

Second reaction: In the presence of lipoprotein lipase (LPL) in Enzyme Solution 2, triglycerides in the sample are rapidly hydrolyzed to glycerol and fatty acids. The glycerol thus formed is converted to glycerol-3-phosphate by the action of GK in the presence of ATP, which is then converted to hydrogen peroxide in the presence of GPO. Hydrogen peroxide causes oxidative condensation of 4-aminoantipyrine and N-Ethyl-N-sulfobutyl-m-toluidine sodium (ESBmT) in the presence of peroxidase (POD) to form a red-purple dye. The triglyceride content is determined by measuring the absorbance of the red-purple dye. The influence of ascorbic acid is blocked by ascorbate oxidase.

First reaction:

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} H_2O_2 + \text{Dihydroxyacetone phosphate}$$

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2$$

Second reaction:

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} H_2O_2 + \text{Dihydroxyacetone phosphate}$$

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

2. Features

1) With this reagent, measurements of TG and other parameters (such as F-CHO, NEFA) is hardly affected even if cross-contamination occurs.
2) Free glycerol is eliminated by the enzymatic method.
3) Bilirubin, hemolysis, ascorbic acid and chyle have minimal effects on results.
4) Applicable to various automated analyzers.

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[1)]
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
Bring samples to room temperature (15–30°C) before use.
3) Measurement of heparinized samples may yield

artificially low values because decomposition of triglycerides is promoted. [5]

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), and ascorbic acid (50 mg/dL).

3. Others

1) Always use Seronorm Lipid, Cholestest N Calibrator or QUALIGENT N Calibrator for Labospect 008 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): TG Enzyme Solution 1 is ready to use.

Reagent (2): TG 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.

Sample 2.4 µL + Reagent (1) 240 µL → (37°C, 5 min) → Measurement (Absorbance I※)

Reagent (2) 80 µL → (37°C, 5 min) → Measurement (Absorbance II※) → Calculation of concentration

※Absorbance I and II: The difference in absorbance between 800 nm and 600 nm.

Calibration material: Seronorm Lipid, Cholestest N Calibrator (manufacture's assigned value), 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[2]

Male: 40–234 mg/dL

Female: 30–117 mg/dL

2. Diagnostic criterion[3]

Hypertriglyceridemia: ≥ 150 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 0.09–0.17 per 200 mg/dL of triglycerides.

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[6]**:** (On Hitachi 7150 automated analyzer)

3–2000 mg/dL

5. Correlation[6]

1) Serum N=60 r=0.999 y=1.04x–0.14
Control method: Approved in vitro diagnostic (enzymatic method)

2) Plasma N=71 r=0.999 y=0.99x–0.46
Control method: Approved in vitro diagnostic (enzymatic method)

6. Standard Material

Reference standard for lipid assay (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 contains human-derived components determined as HBsAg-negative, HIV antibody (AIDS virus antibody) negative, and HCV antibody negative. When using, however, it should be handled very carefully as with samples, considering the risk of infectious.

3) Enzyme Solution 2 contains 0.0005 w/v% sodium azide to inhibit catalase activity. If this solution is accidentally ingested or comes into contact with the eyes or skin, immediately implement first-aid measures such as rinsing the area with water and seek medical treatment if necessary.

4) Proclin 300, which possesses skin-irritative potential, is added as an antiseptic agent in the 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.
5) Enzyme Solution 2 contains 0.0005 w/v% sodium azide, which inhibits the activity of catalase. Sodium azide may react with lead or copper pipes and produce highly explosive metallic azide. When disposing of this product, flush it away with copious amounts of water.

4. Other precautions

Do not use the containers for other purposes.

Storage and Shelf Life

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

Packaging

Name			Package
Cholestest	(1)	Enzyme Solution 1	2 × 400 mL
TG	(2)	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, 263, Kodansha, 1972.
2) Kanai M. (supervising editor): Kanai's manual of clinical laboratory medicine. 34th ed. 514, Kanehara Shuppan, 2015.
3) Japan Atherosclerosis Society, ed. 2012 Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases, 13.
4) Tamura K. et al.: J Med Pharm Sci, 49, 791, 2003.
5) Shirai K.: Modern Med Lab, 21, 3, 211, 1993.
6) In house data, SEKISUI MEDICAL CO., LTD.

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