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

In Vitro Diagnostics
Marketing Approval No. 21200AMZ00383000

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* Revised: April 2008 (6th edition)

This package insert must be read carefully prior to use.

Bile acid assay kit
(Classification No.: 30156000)

Pureauto S TBA

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

TBA Substrate Solution 1:
Oxdized β-thionicotinamide adenine dinucleotide (Thio-NAD)
Good's buffer

TBA Enzyme Solution 2:
Reduced β-Nicotinamide adenine dinucleotide (NADH)
3α-Hydroxysteroid dehydrogenase (3α-HSD)
Good's buffer

Intended Use

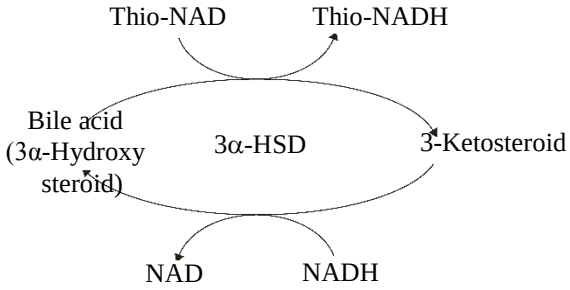
Measurement of bile acid in serum

Bile acid is produced from cholesterol in the liver. Bile acid is mostly secreted into the bile as glycine or taurine conjugates and are stored in the gallbladder. Bile acid stored in the gallbladder is released into the intestine in response to stimulation such as food intake, and promote the digestion and absorption of fats, etc. Then most of the bile acid is reabsorbed in the terminal ileum, transported to the liver via the portal vein, and taken up by hepatocytes (enterohepatic circulation).
Elevation of the concentration of bile acid in the peripheral blood specifically occurs in hepatobiliary diseases. Bile acid levels closely reflect the exacerbation and recovery of these diseases.

Assay Principle

1. Assay Principle

Bile acid in a sample is specifically oxidized by 3α-hydroxysteroid dehydrogenase (3α-HSD) and oxidized β-thionicotinamide adenine dinucleotide (Thio-NAD) to produce 3-ketosteroid and reduced β-thionicotinamide adenine dinucleotide (Thio-NADH). In the presence of 3α-HSD and reduced β-nicotinamide adenine dinucleotide (NADH), reaction with 3-ketosteroid produces bile acid and oxidized β-nicotinamide adenine dinucleotide (NAD). As mentioned above, trace levels of bile acid are amplified by coenzyme cycling, and the change of absorbance resulting from Thio-NADH production is measured to determine the concentration of bile acid.



2. Features

1) Liquid reagents, ready-to-use.
2) A highly sensitive enzyme cycling method is used.
3) The calibration curve is linear up to 180 μmol/L.
4) Applicable to various automated analyzers.

Procedural Precautions ** *

1. Properties of Samples and Sampling Methods

1) Samples
Serum may be used.
Serum bile acid levels are known to increase after meals. Pay attention to the timing of blood collection. If patients are not taking bile acid, collect blood early in the morning after fasting.

2) Storage of samples[4)]
The isolated serum should be tested on the same day.
Store samples at -20°C or lower.
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 100 mg/dL), or formazin turbidity (up to 3000 FTU).

3. Others

1) Always use bile acid standard solution 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): TBA Substrate Solution 1 is ready to use.

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

※After opening, TBA substrate solution 1 and TBA enzyme solution 2 can be used for 1 month if kept in closed containers and stored at 2–10°C.

2. Assay Procedure

This product is compatible with Hitachi 9000 series automated analyzers. An example of the assay procedure is indicated below.

Sample 2.2 µL + Reagent (1) 150 µL → 37°C 5 min → Reagent (2) 50 µL → 37°C 48–186 sec → Measurement (Absorbance※)

→ Calculation of concentration

※ Absorbance: The difference in absorbance between 660 nm and 415 nm

Calibration material: Standard solution of bile acid (Manufacture's assigned value)

Reagent blank: Purified water or saline

Assessment of Assay Results

1. Reference standard range

≤ 10 µmol/L[3)]

2. 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: The change of absorbance is ≤ 0.020/min.

2) Sensitivity: The change of absorbance is 0.020–0.100/ min per 50 µ mol/L of bile acid.

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[5)]: (On Hitachi 7170 automated analyzer)

2–180 µ mol/L

5. Correlatio[5)]

Serum N=69 r=0.999 y=1.01x+0.19

Control method: Approved in vitro diagnostic (enzyme cycling method)

6. Standard Material

Sodium glycochenodeoxycholate (in-house reference standard)

Precautions for Use or Handling **

. 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) The components of this product contain sodium azide as a preservative. If any reagent is accidentally ingested or comes into contact with the eyes or skin, immediately rinse the area with water and seek medical treatment, if necessary.

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 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) The components of this product contains sodium azide as a preservative. 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

1) Do not use the containers for other purposes.

2) Do not take apart the reagent cartridge before use.

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
Pureauto S TBA L set (Set for Hitachi LABOSPECT series)	TBA Substrate Solution 1: 1 × 48 mL	× 2
	TBA Enzyme Solution 2: 1 × 16 mL	

References **

1) Tanaka N. et al.: Jpn J Clin Med, 598 (extra edition), 521–524, 1989.
2) Fujiwara K.: Jpn J Clin Pathol, 37, 10, 1114–1121, 1989.
3) Kanai M. (supervising editor): Kanai's manual of clinical laboratory medicine. 34th ed. 526, Kanehara Shuppan, 2015.
4) Medical Practice Editorial Board: Laboratory test guide 2003–2004, 296, Bunkodo, 2013.
5) 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