(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Total Bile Acid (TBA) Assay Kit

Catalog No.: BC00053

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Total Bile Acid (TBA) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum
Assay Type	Quantitative
Detection Instrument	Microplate reader (400-410 nm, optimal detection wavelength 405 nm）

Product Introduction

Total bile acid is a group of metabolites of cholesterol in the liver and enterohepatic circulation. It is the final product of cholesterol metabolism in the liver and is closely related to the absorption, metabolism and regulation of cholesterol.

Principle

With thio-oxidized coenzyme I (S-NAD^+) as a hydrogen acceptor, 3α-hydroxysteroid dehydrogenase catalyzes the dehydrogenation of bile acid to produce 3-ketosteroids, converting S-NAD^+ into thio-reduced coenzyme I (S-NADH). With reduced coenzyme I (NADH) as a hydrogen donor, 3α-hydroxysteroid dehydrogenase catalyzes 3-ketosteroids to produce bile acid, and through the enzyme cycle reaction, S-NADH is continuously generated, which has a maximum absorption peak at 405 nm.

Components

No.	Components	Size (100T)	Storage
Reagent 1	Color Developer I	20 mL	-20℃, protect from light, store at 2-8°C after opening.
Reagent 2	Color Developer II	6 mL	-20℃, protect from light, store at 2-8°C after opening.
Reagent 3	50μmol/L Standard	0.5 mL	-20℃, store at 2-8°C after opening.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20℃ for 12 months, and after opening, it can be stored at 2-8℃ for 3 months.

Preparation

- **Sample handling**

1. Serum samples: Can be measured directly.
2. Tissue samples: Take 0.020-0.1 g of fresh tissue blocks, rinse with 2-8°C PBS (0.01 M, pH 7.4) to remove blood, blot dry with filter paper, weigh, put into a homogenizer, add physiological saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) at a ratio of weight (g) : volume (mL) = 1:9, and centrifuge at 4°C, 10000×g for 10 min. Take the supernatant and place it on ice for testing, and keep part of the supernatant for protein determination.
3. Sample dilution: Generally, serum samples do not need to be diluted. Before the formal test of special samples, 2-3 samples with large expected differences can be selected and diluted to different concentrations for preliminary experiments. According to the results of the preliminary experiments, combined with the linear range of this kit: 5-120 μmol/L, dilution is performed. The diluent is physiological saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

- **Preparation of the kit**

Take out all reagents and return to room temperature before use.

Operation process

Refer to the table below to set up blank control wells, standard wells, and sample wells on a 96-well plate.

	Blank Control	Standard	Sample
Distilled water	3 μl	--	--
Calibrator	--	3 μl	--
Sample to be tested	--	--	3 μl
Reagent 1	180 μl	180 μl	180 μl

Mix gently by shaking and incubate at 37°C for 5 min.			
Reagent 2	60 μl	60 μl	60 μl
Mix by gentle shaking, incubate at 37°C for 1 min, read the absorbance A1 with a microplate reader at 405 nm, and read the absorbance A2 after incubating at 37°C for 3 min. Calculate ΔA = A2 - A1.			

Calculation

ΔΔA (sample) = ΔA (sample) - ΔA (blank control), ΔΔA (standard) = ΔA (standard) - ΔA (blank control)

TBA content (μmol/L) = ΔΔA (sample) * standard concentration (μmol/L) / ΔΔA (standard)

TBA content in tissue can also be divided by the protein concentration and converted into μmol/g protein.

Notes

1. The optimal detection wavelength of the microplate reader is 405 nm, and detection can be performed in the range of 400 nm-410 nm.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.