

(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 (450 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

Total bile acids in serum are a class of steroid derivatives with a 3 α -hydroxyl group. They undergo oxidation under the action of 3 α -hydroxysteroid dehydrogenase (3 α -HSD). During this process, the coenzyme NAD⁺ is reduced to NADH. Subsequently, in the presence of the electron coupling reagent PMS, WST-8 can be reduced by NADH to produce an orange-yellow formazan, which can be determined colorimetrically at 450 nm. The color intensity is proportional to the concentration of bile acids in the sample.

Components

No.	Components	Size (100T)	Storage
Reagent 1	50mmol/L Standard	1 mL × 1 tube	-20°C, protect from light, store at 2-8°C after opening.
Reagent 2	Color Developer A	1.5 mL × 1 tube	-20°C, protect from light, store at 2-8°C after opening.
Reagent 3	Color Developer B	2.5 mL × 1 tube	-20°C, protect from light, store at 2-8°C after opening.

Reagent 4	Color Developer C	2.5 mL × 1 tube	-20°C, protect from light, store at 2-8°C after opening.
Reagent 5	Buffer	15 mL × 1 bottle	-20°C, store at 2-8°C after opening.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The product should be stored long-term at -20°C. After opening, it can be stored at 4°C for up to 6 months.

Preparation

- **Sample handling**

1. Liquid samples such as serum and plasma: Can be measured directly.
2. Tissue samples: Standard homogenization is performed using PBS (0.01 M, pH 7.4) as the homogenization medium. After homogenization, centrifuge at 10,000×g for 10 min at 4°C. Collect the supernatant and keep on ice until assay.
3. Sample dilution: Prior to formal detection, select 2–3 samples with expected large differences, dilute to various concentrations for pre-experiment. Based on pre-experiment results and the linear range of this kit, determine the appropriate dilution factor.

Diluent: physiological saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

- **Preparation of the kit**

1. Bring all reagents in the kit to room temperature.
2. Preparation of Color Working Solution: Mix Reagent 2, Reagent 3, Reagent 4 and Reagent 5 at a volume ratio of 10:20:20:6. Prepare freshly before use and protect from light during preparation.
3. Dilution of Standards: Dilute the 50 mmol/L standard (Reagent 1) to 50 µmol/L with

double-distilled water, then serially dilute 2-fold with Reagent 5 to obtain 25, 12.5, 6.25 and 0 $\mu\text{mol/L}$ (blank well).

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
Reagent 5 (μL)	60	--	--
Bile acid standards at various concentrations (μL)	--	60	--
Test samples (μL)	--	--	60
Color Working Solution (μL)	100	100	100

Incubate at 37 °C in the dark for 5–10 min. Determine the OD value of each well at 450 nm.

Calculation

Standard Curve Fitting: $y = ax + b$

Calculation of Total Bile Acid Concentration in Normal Serum/Plasma Samples:

$$\text{Total Bile Acid Content } (\mu\text{mol/L}) = (\Delta A_{450} - b) \div a \times f$$

Notes:

y: OD value of standard well – OD value of blank well (OD at 0 standard concentration)

x: Concentration corresponding to absorbance

a: Slope of the standard curve

b: Intercept of the standard curve

ΔA_{450} : OD value of sample – OD value of blank well (OD at 0 standard concentration)

f: Dilution factor of the sample before addition to the assay system

Notes

1. Carefully read the instruction manual and calibrate the instrument before the experiment. Perform the assay strictly in accordance with the manual.
2. Wear a lab coat and latex gloves for protection during the experiment.
3. The detection range of the kit does not equal the concentration range of the analyte in samples. If the analyte concentration in the sample is too high or too low, appropriately dilute or concentrate the sample before testing.
4. If the sample type is not listed in this manual, a preliminary experiment is recommended to verify detection validity.
5. The final experimental results are closely related to reagent validity, operator performance, experimental environment and other factors. Our company is only responsible for the kit itself, and not liable for sample consumption caused by using the kit. Please estimate the required sample volume and reserve sufficient samples before use.
6. This product is for research use only by professional personnel. It is not intended for clinical diagnosis or treatment, not for use in food or pharmaceutical products, and must not be stored in ordinary residential premises.