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

Catalog No：E-BC-K181

Specification：100Assays(Can detect 100 samples with spectrophotometer or 400 samples with biochemical analyzer and microplate reader without duplication)

Measuring instrument：Spectrophotometer, microplate reader, biochemical analyzer

Detection range：0-180 μmol/L

Elabscience® Total Bile Acid (TBA) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com
Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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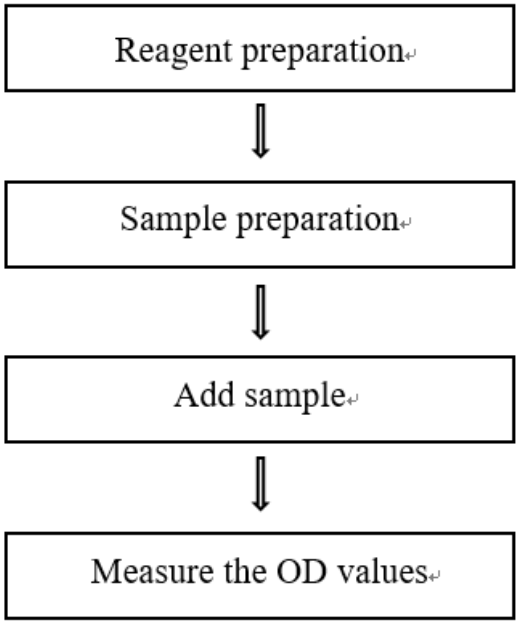
Assay summary



Intended use

The kit is used for the quantitative determination of the total bile acid concentration in serum.

Detection principle

Total bile acid (TBA) is mainly used for the screening and prognosis of follow-up of hepatobiliary disease and as the marker of liver parenchymal damage and cholestasis. The increase of TBA indicates the risk of viral hepatitis, cirrhosis, alcoholic liver disease, drug-induced liver injury or cholestasis.

$$\text{S-NAD} + \text{Bile acid} \xrightarrow{3\alpha-\text{HSD}} \text{3-Ketosteroid} + \text{S-NADH}$$

$$\text{3-Ketosteroid} + \text{S-NADH} \xrightarrow{\text{Diaphorase}} \text{NAD} + \text{Bile acid}$$

Measure the OD value at 405 nm and the changes of absorbance is proportional to the concentration of bile acid.

Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Glycine Auffer	75 mL ×1 vial	2-8℃, 12 months (shading light)
	S-NAD		
	Stabilizer		
Reagent 2	Bile Acidase (3α-HSD)	25 mL ×1 vial	2-8℃, 12 months (shading light)
	NADH		
Reagent 3	50 μmol/L Standard	1 mL ×1 vial	2-8℃, 12months

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Spectrophotometer (405 nm)/Microplate reader (405 nm)/Biochemical analyzer (405 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

Equilibrate all the reagents to room temperature before use.

Sample preparation

1. Separate serum within 2 hours after blood collection. The serum sample can be stored at 15~30℃ within 8 hours, at 2~8℃ for a week or at -20℃ for 3 months.
2. Interfering substances: conjugated bilirubin ≤ 5mg/dL, unconjugated bilirubin ≤ 20mg/dL, vitamin C ≤ 1mg/dL, triglyceride ≤ 9.25 mmol/L, hemoglobin ≤ 100mg/dL have no effect to the results.

The key points of the assay

① The sample needs to be diluted with normal saline before the determination when the concentration of TBA is higher than 180 μmol/L. The result should be multiplied by the dilution factor.

② The kit is for research use only and contains preservatives. It should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.

③ The ratio of sample and reagent can be scaled as required.

④ The reaction time can be prolong to 5 min or 10 min from 3 min if the △A is less than 0.003.

Operating steps

1. Detection with spectrophotometer

	Blank tube	Standard tube	Sample tube
Double distilled water (μL)	10		
Standard (μL)		10	
Sample (μL)			10
Reagent 1 (μL)	720	720	720
Mix fully and incubate at 37℃ for 5 min.			
Reagent 2 (μL)	240	240	240
Mix fully and incubate at 37℃ for 1 min. Set spectrophotometer to zero with double distilled water and measure the absorbance at 405 nm at 0 second (A_1) and 3 min (A_2), respectively. Calculate the $\triangle A=A_2-A_1$.			

2. Detection with microplate reader

	Blank tube	Standard well	Sample well
Double distilled water (μL)	2.5		
Standard (μL)		2.5	
Sample (μL)			2.5
Reagent 1 (μL)	180	180	180
Mix fully and incubate at 37℃ for 5 min.			
Reagent 2 (μL)	60	60	60
Mix fully and incubate at 37℃ for 1 min. Measure the absorbance at 405 nm at 0 second (A_1) and 3 min (A_2), respectively. Calculate the $\triangle A=A_2-A_1$.			

3. Detection with biochemical analyzer

Temperature	37℃	Method	Two-point end point method
Dominant wavelength	405 nm	Optical path	1 cm
Reaction direction	Up	Sample	2.5 μL
Reagent 1	180 μL	Reagent 2	60 μL
Incubation time (Sample+ Reagent 1)	5 min		
Incubation time (Sample+ Reagent 1+ Reagent 2)	1 min		

Measure the absorbance at 0 second (A_1) and 180 second (A_2), respectively.

Calculate the $\triangle A=A_2-A_1$.

Automatic biochemical analyzer has its own program parameter input language. Reagents matches the analyzer and carry out automatic measurement after the above basic parameters are modified.

Calculation

The sample:

$$\text{TBA content (μmol/L)} = \frac{\triangle A_{Sample}-\triangle A_{Blank}}{\triangle A_{Standard}-\triangle A_{Blank}} \times c \times f$$

[Note]

c: Concentration of standard.

f: Dilution factor of the sample before tested.

Performance index

① Linear range: 0-180 μmol/L, $r^2 \geq 0.990$.

② Accuracy: inaccuracy ≤ 15.0%.

③ Recovery rate: 100 ±20%

④ Precision: intra-CV ≤ 5.0%, inter-CV ≤ 10.0%.

⑤ Absorbance for the blank control (reagents only) ≤ 0.7 (405 nm wavelength, 1 cm optical path).

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.