Total Bile Acid (TBA) Colorimetric Assay Kit

Catalog No: E-BC-K181

Method: Colorimetric method

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

Instrument: Spectrophotometer, microplate reader, biochemical analyzer

Detection range: 0-180 μmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

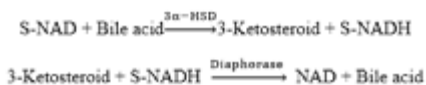
▲Intended use

The kit is used for the quantitative determination of the total bile acid (TBA) content in serum.

▲Background

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.

▲Detection principle



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 buffer	75 mL × 1 vial	2-8℃ , 6 months
	S-NAD		
	Stabilizer		
Reagent 2	Bile acidase (3α-HSD)	25 mL × 1 vial	2-8℃ , 6 months, shading light
	NADH		
Reagent 3	50 μmol/L Standard	1 mL × 1 vial	2-8℃ , 6 months, shading light
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.			

▲Materials prepared by users

Instruments

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

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

Pre-assay preparation

▲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.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	405 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operation table

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 (A1) and 3 min (A2), respectively. Calculate the ΔA=A2-A1.			

2. Detection with microplate reader

	Blank tube	Standard tube	Sample tube
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 (A1) and 3 min (A2), respectively. Calculate the ΔA=A2-A1.			

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 (A1) and 180 second (A2), respectively. Calculate the ΔA=A2-A1.

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

$$TBA(\mu mol/L) = \frac{\Delta A_{Sample}-\Delta A_{Blank}}{\Delta A_{Standard}-\Delta A_{Blank}} \times c \times f$$

Note:

c: Concentration of standard.

f: Dilution factor of sample before test.

▲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, 1 cm optical path).

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.
5. 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.
6. 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.
7. The ratio of sample and reagent can be scaled as required.
8. The reaction time can be prolong to 5 min or 10 min from 3 min if the ΔA is less than 0.003.