Technical Manual

Total Bile Acid (TBA) Colorimetric Assay Kit

- Catalogue Code: MAES0134
- Size: 100 Assays
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0-180 µmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. Detection Principle

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

5. Kit Components & Storage

Item	Specification	Storage
Working solution 1	75 mL × 1 vial	2-8°C, 6 months
Working solution 2	25 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard	1 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (405 nm)/Microplate reader (405 nm)/Biochemical analyzer (405 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)

6. Assay Notes:

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

2. 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.

7. Sample Preparation

1. Separate serum within 2 hours after blood collection. The serum sample can be stored at 15~30°C within 8 hours, at 2~8°C for a week or at -20°C 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.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0-180 μmol/L).

8. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 405 nm

9. Operation Steps

Operation Table (Detection with Biochemical analyser)

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

Operation Table (Detection with spectrophotometer)

	Blank tube	Standard tube	Sample tube
Double distilled water (μL)	10		
Standard (μL)		10	
Sample (μL)			10
Working solution 1 (μL)	720	720	720
Mix fully and incubate at 37°C for 5 min.			
Working solution 2 (μL)	240	240	240
Mix fully and incubate at 37°C 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 $\triangle A=A_2-A_1$.			

Operation Table (Detection with microplate reader)

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

10. Calculations

$$TBA\ content\ \left(\frac{\mu mol}{L}\right) = \frac{\triangle A_{Sample} - \triangle A_{Blank}}{\triangle A_{Standard} - \triangle A_{Blank}} \times \text{Concentration of standard } (\mu mol/L)$$

11. Performance Characteristics

Detection Range	0-180 µmol/L

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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