Technical Manual

Total Bile Acid (TBA) Colorimetric Assay Kit

- Catalogue Code: MAES0135
- Size: 96T
- Research Use Only

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

1.77-40 μmol/L

Sensitivity:

1.36 μmol/L

Storage:

2-8°C for 3 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 3 months.

Do not use components from different batches of kit.

2. Background:

Bile acids usually consist of a 24-carbon steroid core and a side chain with a carboxyl group. The catabolites of mammalian cholesterol are primarily primary bile acids, including cholic and deoxycholic acids, which are secreted into bile in combination with taurine or glycine. The primary bile acids are dehydroxylated and converted to secondary bile acids, including deoxycholic and lithocholic acids. Metabolism disorder of bile acid is related to liver disease, inflammatory bowel disease, non-alcoholic fatty liver disease, diabetes, obesity and other diseases.

3. Intended Use:

The kit can be used to detect the concentration of total bile acid (TBA) in serum samples.

4. Detection Principle:

With S-NAD+ as hydrogen receptor, 3α-hydroxy steroid dehydrogenase catalyzed the dehydrogenation of bile acids to produce 3-ketone steroids, transforming S-NAD+ into S-NADH. Meanwhile, NADH was used as hydrogen donor. 3α-hydroxy steroid dehydrogenase catalyzed the production of bile acids from 3-ketone steroids. Through the enzyme cycle reaction, S-NADH is continuously generated, which has the maximum absorption peak at 405 nm. 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
Chromogenic Agent A	24 mL × 1 vial	2-8°C, 3 months, avoid direct sunlight
Chromogenic Agent B	6 mL × 1 vial	2-8°C, 3 months, avoid direct sunlight
Standard (0.2 mmol/L)	2 mL × 1 vial	2-8°C, 3 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (400-410 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 supernatant must be clarified.

2. Prevent the formation of bubbles when the reagent or sample is transferred into the microplate.

7. Reagent Preparation:

Bring all reagents to room temperature before use.

8. Sample Preparation:

Serum sample:
Fresh blood was collected and placed at 25°C for 30 min to clot the blood. Centrifuge the sample at 4°C for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection. If not detected on the same day, stored the serum at -80°C, which can be stored for a month.

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 (1.77-40 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
Human serum	1
Dog serum	1
Mouse serum	1
Rat serum	1
Bovine serum	1

Note: The diluent is normal saline;

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 405 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps:

The preparation of standard curve

Dilute 0.2 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 25, 30, 35, 40 μmol/L.

The measurement of samples

1. **Standard well:** add 10 μL of standards with different concentrations to the corresponding wells.
   Sample well: add 10 μL of sample to the corresponding wells.

2. Add 200 μL of chromogenic agent A to each well.

3. Add 50 μL of chromogenic agent B to each well.

4. Mix fully and incubate at 37°C for 3 min.

5. Measure the absorbance of each well at 405 nm, recorded as A_1.

6. Incubate at 37°C for 5 min.

7. Measure the absorbance of each well at 405 nm, recorded as A_2. Calculate the △A/min = $(A_2-A_1)/5$.

Operation Table

	Standard well	Sample well
Standard with different concentrations (μL)	10	
Sample (μL)		10
Chromogenic agent A	200	200
Chromogenic agent B	50	50
Mix fully and incubate at 37°C for 3 min. Measure the absorbance of each well at 405 nm, recorded as A_1. Incubate at 37°C for 5 min. Measure the absorbance of each well at 405 nm, recorded as A_2. Calculate the △A/min = $(A_2-A_1)/5$.		

11. Calculations:

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

$$\text{TBA content (μmol/L)} = (\Delta A_{405} - b) \div a \times f$$

y: $\Delta A_{Standard/min} - \Delta A_{Blank/min}$ ($\Delta A_{Blank/min}$ is the ΔA/min value when the standard concentration is 0)
x: The concentration of standard
a: The slope of standard curve
b: The intercept of standard curve
ΔA_{405}: $\triangle A_{Sample/min} - \triangle A_{Blank/min}$
f: Dilution factor of the sample before test

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12. Performance Characteristics:

Detection Range	1.17-40 μmol/L
Sensitivity	1.36 μmol/L
Average recovery rate (%)	97
Average inter-assay CV (%)	3.7
Average intra-assay CV (%)	3.4

Analysis

Take 10 μL of human serum, carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.00259 x + 0.00035, the average A_1 of the sample is 0.390, the average A_2 of the sample is 0.615, the average A_1 of the blank is 0.349, the average A_2 of the blank is 0.349, $\triangle A_{Sample}$/min=(0.615-0.390)/5=0.045, $\triangle A_{Blank}$/min=(0.349-0.349)/5=0, and the calculation result is:

TBA content (μmol/L) = (0.045-0.00035) ÷ 0.00259

= 17.24 μmol/L

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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