Total Bile Acid (TBA) Colorimetric Assay Kit

Catalog No: E-BC-K181-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 2.05 μmol/L

Detection range: 2.05-120 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 can be used to detect the concentration of total bile acid (TBA) in serum samples.

▲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.

▲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.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Chromogenic Agent A	24 mL × 1 vial	2-8℃ , 3 months, shading light
Reagent 2	Chromogenic Agent B	6 mL × 1 vial	2-8℃ , 3 months, shading light
Reagent 3	0.2 mmol/L Standard	2 mL × 1 vial	2-8℃ , 3months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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

Microplate reader (400-410 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

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.

▲The key points of the assay

1. The supernatant of sample must be clarified.
2. Prevent the formulation of bubbles when the reagent or sample is transferred into the microplate.

Pre-assay preparation

▲Sample preparation

Serum sample:

Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4℃ 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℃ , which can be stored for a month.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (2.05-120 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 (0.9% NaCl).

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

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

▲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.

▲Operating 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, 0.01, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12 mmol/L.

The measurement of samples

1) Standard well: add 3 μL of standard with different concentrations to the corresponding wells.
   Sample well: add 3 μL of sample to the corresponding wells.
2) Add 200 μL of reagent 1 to each well.
3) Add 50 μL of reagent 2 to each well.
4) Mix fully and incubate at 37℃ for 1.5 min.
5) Measure the absorbance of each well at 405 nm, recorded as A_1.
6) Incubate at 37℃ for 2 min.
7) Measure the absorbance of each well at 405 nm, recorded as A_2. Calculate the $\Delta A/min = (A_2-A_1)/2$.

▲Operation table

	Standard well	Sample well
Standard with different concentrations (μL)	3	
Sample (μL)		3
Reagent 1 (μL)	200	200
Reagent 2 (μL)	50	50
Mix fully and incubate at 37℃ for 1.5 min. Measure the absorbance of each well at 405 nm, recorded as A_1. Incubate at 37℃ for 2 min. Measure the absorbance of each well at 405 nm, recorded as A_2. Calculate the $\Delta A/min = (A_2-A_1)/2$.		

Calculation

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.

$$\text{TBA content (μmol/L)} = (\Delta A_{405} - b) \div a \times 1000^{*} \times f$$

Note:

y: $\Delta A_{Standard}$/min –ΔA_{Blank}/min (ΔA_{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.

ΔA405: ΔA_{Sample}/min-ΔA_{Blank}/min

1000*: 1 mmol/L=1000 μmol/L

f: dilution factor of the sample before tested

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 3 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	2.05-120 μmol/L	Average intra-assay CV (%)	3.4
Sensitivity	2.05 μmol/L	Average inter-assay CV (%)	3.7
Average recovery rate (%)	97		

▲Example analysis

Take 3 μL of human serum and carry the assay according to the operation table. The results are as follows:

Standard curve:y = 0.9465 x + 0.0005, the average A_1 of the sample is 0.404, the average A_2 of the sample is 0.438, the average A_1 of the blank is 0.353, the average A_2 of the blank is 0.353, ΔA_{Sample}/min=(0.438-0.404)/2=0.017, ΔA_{Blank}/min=(0.353-0.353)/2=0, and the calculation result is:

$$\text{TBA content (μmol/L)} = \frac{0.017 - 0.0005}{0.9465} \times 1000 = 17.43\ \text{μmol/L}$$