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

Catalog No：E-BC-K181-M

Specification：48T(32 samples)/96T(80 samples)/ 500Assays(484 samples)

Measuring instrument：Microplate reader（400-410 nm）

Detection range：1.77-40 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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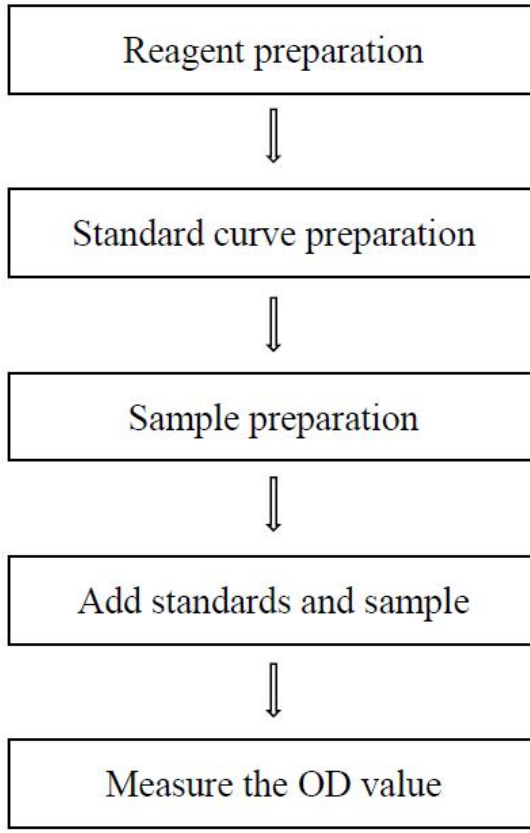
Assay summary



Intended use

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

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	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500Assays)	Storage
Reagent 1	Chromogenic Agent A	12 mL×1 vial	24 mL×1 vial	60 mL×2 vials	2-8°C, 12 months, shading light
Reagent 2	Chromogenic Agent B	3 mL×1 vial	6 mL×1 vial	30 mL×1 vial	2-8°C, 12 months, shading light
Reagent 3	0.2 mmol/L Standard	2 mL×1 vial	2 mL×1 vial	10 mL×1 vial	2-8°C, 12 months
	Microplate	48 wells	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (400-410 nm, optimum wavelength: 405 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**20**	**25**	**30**	**35**	**40**
0.2 mmol/L standard (μL)	0	25	50	100	125	150	175	200
Double distilled water (μL)	1000	975	950	900	875	850	825	800

Sample preparation

① Sample preparation:

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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
10% mouse liver	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M，pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

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.

Operating steps

① Standard well: add 10 μL of standard with different concentrations to the corresponding wells.

Sample tube: 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 $\triangle A/min = (A_2-A_1)/5$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean ΔA_{Blank}/min (ΔA/min value when the standard concentration is 0) of the blank (Standard #①) from all standard readings. This is the ΔA_{405} ($\Delta A_{Standard}$/min – ΔA_{Blank}/min).

3. Plot the standard curve by using ΔA_{405} of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

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

2. Animal tissue sample:

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

[Note]

ΔA_{405}: $\triangle A_{Sample}$/min-$\triangle A_{Blank}$/min

f: Dilution factor of the sample before tested.

C_{pr}: The concentration of protein in sample, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	5.60	13.50	34.00
%CV	3.7	3.4	3.1

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	5.60	13.50	34.00
%CV	3.1	3.8	4.2

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 97%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	8.5	22	33.5
Observed Conc. (μmol/L)	8.6	20.7	32.2
Recovery rate (%)	101	94	96

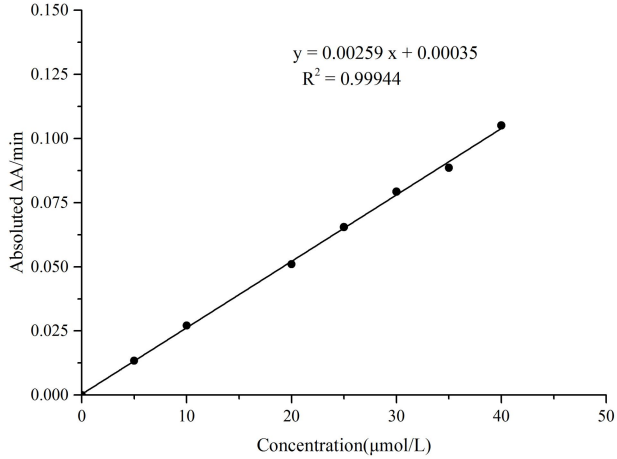
Sensitivity

The analytical sensitivity of the assay is 1.36 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

Concentration (μmol/L)	**0**	**5**	**10**	**20**	**25**	**30**	**35**	**40**
A_1 value	0.348	0.384	0.421	0.487	0.530	0.578	0.607	0.644
	0.349	0.384	0.422	0.492	0.535	0.575	0.604	0.648
Average A_1	0.349	0.384	0.422	0.490	0.533	0.577	0.606	0.646
A_2 value	0.350	0.452	0.557	0.743	0.850	0.978	1.044	1.166
	0.350	0.452	0.559	0.748	0.872	0.970	1.055	1.179
Average A_2	0.350	0.452	0.558	0.746	0.861	0.974	1.050	1.173
$\Delta A/min = (A_2-A_1)/5$	0.000	0.014	0.027	0.051	0.066	0.080	0.089	0.105
Absoluted ΔA/min	0.000	0.013	0.027	0.051	0.066	0.079	0.089	0.105



Appendix II Example Analysis

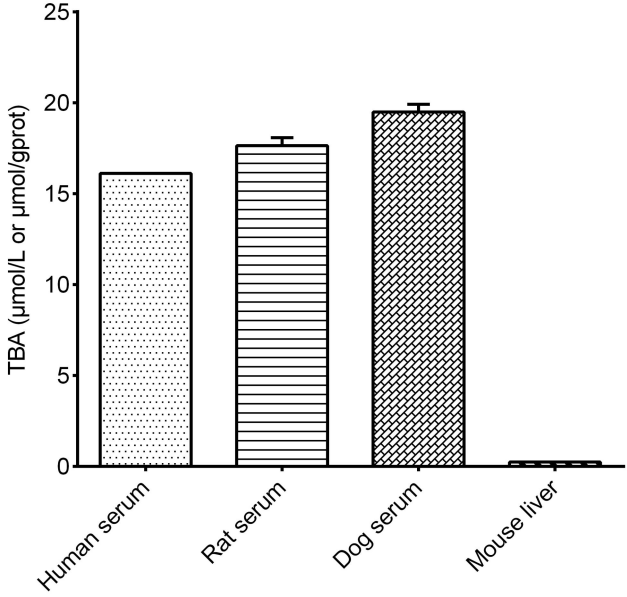
Example analysis:

Take 10 μL of human serum and carry the assay according to the operation steps. 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:

$$\text{TBA content (μmol/L)} = (\Delta A_{405} - b) \div a \times f = (0.045\text{-}0.00035) \div 0.00259 = 17.24 \text{ μmol/L}$$

Detect human serum, rat serum, dog serum and 10% mouse liver tissue homogenate (the concentration of protein is 7.02 gprot/L) according to the protocol, the result is as follows:



Appendix III Publications

1. Wang Y, Wang J, Zhou T , et al.Investigating the potential mechanism and therapeutic effects of SLXG for cholesterol gallstone treatment[J]. Phytomedicine, 2024, 132(000):16. DOI:10.1016/j.phymed. 2024.155886.
2. Ma X, Zhang W, Chen Y, et al. Paeoniflorin inhibited GSDMD to alleviate ANIT-induced cholestasis via pyroptosis signaling pathway[J]. Phytomedicine, 2024, 134(000):11. DOI: 10.1016/j.phymed. 2024.156021.
3. Zhang X, Li Z, Hu R,et al. Exposure memory and susceptibility to ambient PM2.5: A perspective from hepatic cholesterol and bile acid metabolism[J]. Ecotoxicology and Environmental Safety, 2024, 280(000):10. DOI:10.1016/j.ecoenv. 2024.116589.
4. Han S, Wang K, Shen J, et al. Probiotic Pediococcus pentosaceus Li05 Improves Cholestasis through the FXR-SHP and FXR-FGF15 Pathways[J]. Nutrients, 2023, 15(23):16. DOI: 10.3390/nu15234864.
5. Wu L, Li W, Chen G, et al. Ameliorative effects of monascin from red mold rice on alcoholic liver injury and intestinal microbiota dysbiosis in mice[J]. Food Bioscience, 2022. DOI: 10.1016/j.fbio.2022.102079.

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.