TECHNICAL MANUAL

Total Bile Acid (TBA) Colorimetric Assay Kit

- **SKU CODE:** MAES0135
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Equilibrate reagents and prepare standards
- Prepare samples
- Add standards and samples to microplate
- Add chromogenic agents A and B
- Incubate and measure absorbance
- Calculate concentration using standard curve

2. Intended use

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

3. Detection principle

With S-NAD+ as the hydrogen receptor, 3α-hydroxy steroid dehydrogenase catalyzes the dehydrogenation of bile acids to produce 3-ketone steroids, transforming S-NAD+ into S-NADH. Meanwhile, NADH is used as a hydrogen donor, and 3α-hydroxy steroid dehydrogenase catalyzes the production of bile acids from 3-ketone steroids. Through this enzyme cycle reaction, S-NADH is continuously generated, which has a maximum absorption peak at 405 nm. The OD value is measured at 405 nm, and the changes in absorbance are proportional to the concentration of bile acid.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500Assays)	Storage
Reagent 1	Chromogenic Agent A	12 mL x 1 vial	24 mL x 1 vial	60 mL x 2 vials	2-8°C, 12 months, shading light
Reagent 2	Chromogenic Agent B	3 mL x 1 vial	6 mL x 1 vial	30 mL x 1 vial	2-8°C, 12 months, shading light
Reagent 3	0.2 mmol/L Standard	2 mL x 1 vial	2 mL x 1 vial	10 mL x 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	/	No requirement

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500Assays)	Storage
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. 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)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **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 to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 25, 30, 35, 40 μmol/L.

7. Sample preparation

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 10,000 x 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.

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only): 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 dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The supernatant of the sample must be clarified.

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

9. Operating steps

1. Standard well: Add 10 μL of standard 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 A1.

6. Incubate at 37°C for 5 min.

7. Measure the absorbance of each well at 405 nm, recorded as A2.

8. Calculate the ΔA/min = (A2-A1)/5.

10. Calculation

The standard curve:

1. Average the duplicate readings 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 (ΔA_Standard/min – ΔA_Blank/min).

3. Plot the standard curve 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:

TBA content (μmol/L) = (ΔA_405 - b) ÷ a × f

2. Animal tissue sample:

TBA content (μmol/gprot) = (ΔA_405 - b) ÷ a × f ÷ C_{pr}

[Note]

ΔA_405: ΔA_Sample/min - ΔA_Blank/min

f: Dilution factor of the sample before tested.

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

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Inter-assay Precision

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

Recovery

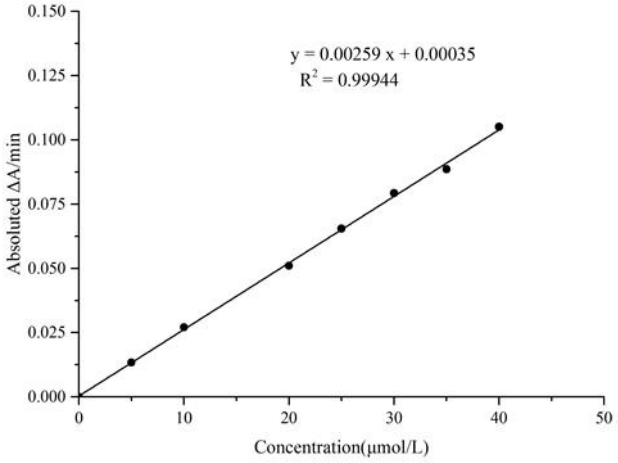
Three samples of high, middle, and low concentrations were tested with each concentration measured 6 times in parallel to achieve an average recovery rate of 97%.

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.

Standard curve data

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), the standard curve and data are provided below for reference only.



12. Appendix II Example Analysis

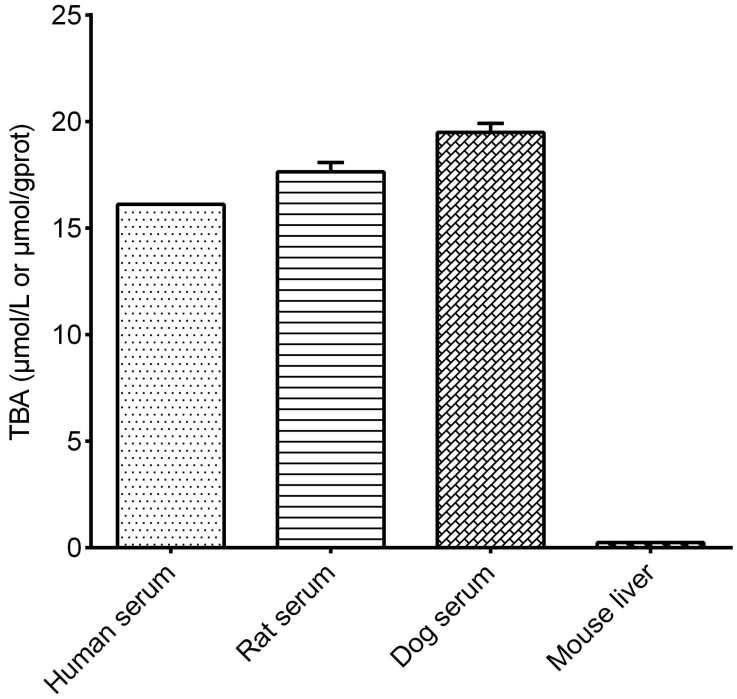
Example analysis:

Take 10 μL of human serum and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.00259x + 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, ΔA_Sample/min = (0.615 - 0.390) ÷ 5 = 0.045, ΔA_Blank/min = (0.349 - 0.349) ÷ 5 = 0, and the calculation result is:

TBA content (μmol/L) = (ΔA_405 - b) ÷ a × f = (0.045 - 0.00035) ÷ 0.00259 = 17.24 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 results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.