Technical Bulletin

Total Bile Acids (TBA) Assay Kit (Colorimetric)

Catalog Number MAK382

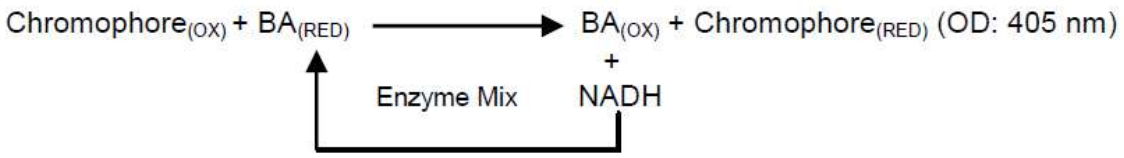
Product Description

Bile Acids (BA) make up 67% of the total composition of Bile. They are 24-carbon steroids generated during cholesterol metabolism and form conjugates with either glycine or taurine to form bile salts. Bile acids are critical due to their ability to solubilize lipids by forming micelles with cholesterol, and fatty acids. Their synthesis is not only necessary for the removal of cholesterol from the body, but they are also needed for proper uptake of dietary lipids into the small intestine. The measurement of circulatory Total Bile Acids (TBA) provides information about hepatic functions and liver diseases such as jaundice, and hepatocellular injury. TBA estimation can detect liver damage during early stages and permits patients to get treatment before hepatic damages become irreversible. In addition, Bile Acids participate as signaling molecules interacting with G-protein coupled receptors (GPCR), TGR5, and nuclear receptor farnesoid X receptor (FXR).

The Total Bile Acid Assay Kit provides a simple and sensitive method to detect physiological concentrations of total bile acids in a variety of biological fluids. The principle of the assay is based on an enzymatic cycling method in the presence of NADH and a chromophore. The reduction of the chromophore produces a stable colorimetric product which can be measured kinetically at 405 nm. This absorbance is directly proportional to the amount of TBA in the sample.

The assay is highly specific and sensitive and is suitable for high-throughput applications. The method can detect as little as 1 mM of Bile Acids.

The kit is suitable for the determination of bile acids in biological fluids such as serum, plasma, bile, urine, saliva, etc. The kit measures total bile acids and cannot distinguish between the various types.



Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- TBA Cycling Assay Buffer 7 mL
  Catalog Number MAK382A
- TBA Probe Buffer 14 mL
  Catalog Number MAK382B
- TBA Probe 1 vial
  Catalog Number MAK382C
- TBA Cycling Enzyme Mix 1 vial
  Catalog Number MAK382D
- NADH 1 vial
  Catalog Number MAK382E
- TBA Standard (100 mM) 1 vial
  Catalog Number MAK382F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well flat-bottom plate. It is recommended to use clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

TBA Cycling Assay Buffer and TBA Probe Buffer: Store at -20 °C or 2-8 °C. Warm to room temperature prior to use.

TBA Probe: Reconstitute with 220 µL of TBA Probe Buffer. Protect from light. Aliquot and store at -20 °C. Bring to room temperature prior to use.

TBA Cycling Enzyme Mix: Reconstitute with 220 µL of TBA Cycling Assay Buffer. Aliquot and store at -20 °C, protected from light. Freeze/thaw cycles should be limited to two. Keep on ice during use.

NADH: Reconstitute with 220 µL of purified water. Aliquot and store at -20 °C, protected from light. Freeze/thaw cycles should be limited to two. Keep on ice during use. Use within two months of reconstitution.

TBA Standard: Reconstitute with 100 µL of purified water to generate a 100 mM Bile Acids Standard. Dissolve completely. Store at -20 °C. Use within two months of reconstitution.

Procedure

Sample Preparation

Notes:

a) Bile Acids concentrations vary over a wide range depending on the sample. TBA range concentrations in some biological samples are:
   - Human serum: <10 µM
   - Human urine (adult): 0-30 µmol/mmol Creatinine
   - Saliva: 0-5 µM

   For unknown Samples (S), perform a pilot experiment by testing several dilutions to ensure the readings are within the Standard Curve range.

b) Metabolites found in biological samples may interfere with the assay. If interference is observed in the diluted samples, prepare parallel sample well(s) as Sample Background Control(s) (SBC) and bring to a total volume of 50 µL/well with purified water.
c) To ensure accurate determination of Bile Acids in the test samples or for samples having low concentrations of Bile Acids, it is recommended to include duplicated samples spiked with a known amount of TBA Standard at a concentration of 0.072 nmol. Add 6 µL of the 12 µM Bile Acids Standard solution (see below) to the duplicated sample well for use as the Spiked Sample (SS).

Serum and urine samples can be assayed directly. Add 5-50 µL of undiluted sample to a 96-well plate.

For ALL Sample (S) and Spiked Sample (SS) wells, adjust the total volume to 50 µL per well with purified water.

Standard Curve Preparation

Note: The assay measures enzymatic activity rates (Abs/min). For maximum accuracy, it is recommended to measure the standard curve and sample wells at the same time.

1. Prepare a 1 mM Bile Acids Standard solution by diluting 10 µL of TBA Standard (100 mM) with 990 µL of purified water.
2. Prepare a 12 µM Bile Acids Standard solution by adding 12 µL of 1 mM Bile Acids Standard solution from Step 1 to 988 µL of purified water.
3. Prepare Bile Acids Standards in desired wells of a clear flat-bottom 96-well plate according to Table 1.

Table 1.
Preparation of Bile Acids Standards

Well	12 µM Bile Acids Standard	Purified Water	Bile Acids (pmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	24
3	4 µL	46 µL	48
4	6 µL	44 µL	72
5	8 µL	42 µL	96
6	10 µL	40 µL	120

50× TBA Probe Mix Preparation

Mix enough reagents for the number of assays to be performed. For each well, prepare 100 µL of 50× TBA Probe Mix according to Table 2.

Table 2.
Preparation of 50× TBA Probe Mix

Reagent	Volume
TBA Probe Buffer	98 µL
TBA Probe	2 µL

Reaction Mix Preparation

1. Mix enough reagents for the number of assays to be performed.
   a. For each Sample (S), Spiked Sample (SS), and Standard well, prepare 50 µL of Sample Reaction Mix according to Table 3. Mix well.
   b. For each Sample Background Control (SBC) well, prepare 50 µL of Sample Background Control Reaction Mix according to Table 4. Mix well.

Table 3.
Preparation of Sample Reaction Mix

Reagent	Volume
TBA Cycling Assay Buffer	46 µL
TBA Cycling Enzyme Mix	2 µL
NADH	2 µL

Table 4.
Preparation of Sample Background Control Reaction Mix

Reagent	Volume
TBA Cycling Assay Buffer	48 µL
NADH	2 µL

Assay Procedure

1. Add 100 µL of 50× TBA Probe Mix to each Standard, Sample Background Control (SBC), Sample (S) and Spiked Sample (SS) wells.
2. Incubate for 10 minutes at 37 °C.
3. Add 50 µL of Sample Reaction Mix to each Standard, Sample (S) and Spiked Sample (SS) well and mix well.
4. Add 50 µL of Sample Background Control Reaction to each Sample Background Control (SBC) well and mix well.

Measurement

Measure absorbance at 405 nm (A_{405}) in kinetic mode at 37 °C for 60 minutes, protected from light.

Results

1. Choose two time points (T_1 and T_2) in the linear range to calculate the slope of every assayed well. Slopes for Standards, Sample Background Controls (SBC), Samples (S), and Spiked Samples (SS) should be calculated using the same time points.
2. Subtract 0 TBA Standard slope value from all Standard slope values.
3. Plot the TBA Standard Curve.
4. If the Sample Background Control (SBC) slope value is significant, then subtract the Sample Background Control (SBC) slope value from Sample (S) and Spiked Sample (SS) slope values to obtain corrected slope values (Sample Slope $_{Corrected}$ and Spiked Sample Slope $_{Corrected}$).
5. Apply corrected Sample (S) slope value (Sample Slope $_{Corrected}$) to Standard Curve to calculate B nmol TBA in the Sample well.

   Sample TBA Concentration (nmol/µL or mM) =

   $$(B/V) \times D$$

   where:

   B = Amount of TBA in the sample well from Standard Curve (nmol)

   V = Sample volume added into the reaction well (µL)

   D = Sample dilution factor (D=1 when samples are undiluted)

6. For Spiked Samples, correct for Sample interference by subtracting the Sample (S) slope values from the Spiked Sample (SS) slope values:

   TBA amount (B) in Sample for Spiked Sample (in nmol) =

$$\left[\frac{\text{Sample Slope}_{Corrected}}{\text{Spiked Sample Slope}_{Corrected} - \text{Sample Slope}_{Corrected}}\right] \times 0.072$$

where:

0.072 = Amount of TBA added to the Spiked Sample (in nmol)

Figure 1.
Typical Total Bile Acids Standard Curve

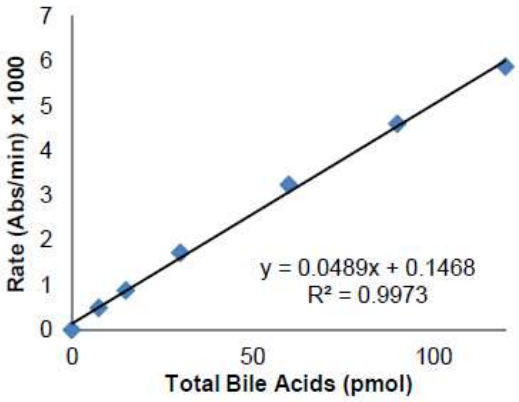
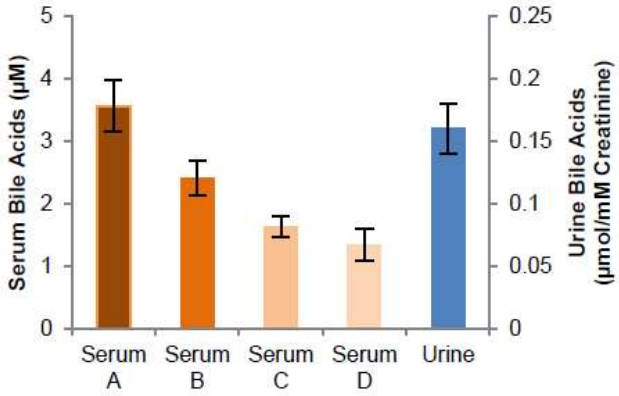


Figure 2.
Estimation of Bile Acids concentration in human serum and urine. 30 µL of each undiluted sample was assayed following the kit protocol. Bile Acids concentrations are:
Serum (in µM): A: 3.56 ± 0.41,
B: 2.41 ± 0.27, C: 1.63 ± 0.17,
D: 1.34 ± 0.25,
Urine: 0.16 ± 0.02 µM/mM Creatinine.



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