Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

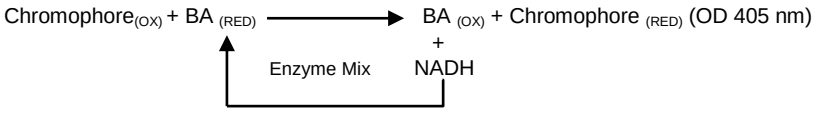
Total Bile Acids (TBA) Assay Kit (Colorimetric)

rev 06/21

(Catalog # K209-100; 100 assays; Store at -20 °C)

I. Introduction:

Bile Acids (BA) make 67% of the total composition of Bile. They are 24-carbon steroids generated during cholesterol metabolism. They form conjugates with either glycine or taurine to form bile salts. Five of the BAs account for more than 99% of the total population found in biofluids. The average composition in healthy individuals includes conjugates of cholic, chenodeoxycholic, deoxycholic and lithocholic acids. Bile acids are critical due to their ability to solubilize lipids by forming micelles with cholesterol, and fatty acids. Their synthesis is not only critical for the removal of cholesterol from the body abut they are also needed for proper uptake of dietary lipids into the small intestine. The measurement of circulatory Total Bile Acids (TBA) therefore 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). **BioVision's Total Bile Acid Assay Kit** provides a simple, sensitive, and high-throughput adaptable approach to detect physiological concentration 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 the absorbance of which can be followed kinetically at 405 nm. This absorbance is directly proportional to the amount of TBA in the sample. Our assay is very specific and sensitive. The assay can detect as little as 1 µM of Bile Acids in a variety of samples.



II. Application:

- Estimation of Bile Acids in various biological samples.

III. Sample Type:

- Biological fluids such as serum, plasma, bile, urine, saliva, etc.

IV. Kit Contents:

Components	K209-100	Cap Code	Part Number
TBA Cycling Assay Buffer	7.0 ml	NM	K209-100-1
TBA Probe Buffer	14 ml	WM	K209-100-2
TBA Probe	1 vial	Red	K209-100-3
TBA Cycling Enzyme Mix	1 vial	Green	K209-100-4
NADH	1 vial	Blue	K209-100-5
TBA Standard (100 mM)	1 vial	Yellow	K209-100-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.

- **TBA Cycling Assay Buffer and TBA Probe Buffer**: Store at -20 °C or 4 °C. Bring to room temperature (RT) before use.
- **TBA Probe:** Reconstitute with 220 µl TBA Probe Buffer. Protect from light. Aliquot and store at -20 °C. Bring to RT before use.
- **TBA Cycling Enzyme Mix:** Reconstitute with 220 µl TBA Cycling Assay Buffer. Aliquot and store at -20 °C. Protect from light. Freeze/thaw should be limited to two times. Keep on ice during use.
- **NADH:** Reconstitute with 220 µl of ddH_2O. Aliquot and store at -20 °C. Protect from light. Freeze/thaw should be limited to one time. Keep on ice during use. Use within 2 months.
- **TBA Standard:** Reconstitute with 100 µl of ddH_2O to generate 100 mM Bile Acids Standard. Dissolve completely. Store at -20 °C. Use within 2 months.

VII. Total Bile Acids Assay Protocol:

1. **Sample Preparation:** Serum and urine samples can be assayed directly. Add 5-50 µl undiluted sample to a 96-well plate. Adjust the volume to 50 µl/well with ddH_2O.

   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, we recommend doing a pilot experiment & testing several doses 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) and make up the volume to 50 µl/well ddH_2O.

   c. To ensure accurate determination of Bile Acids in the test samples or for samples having low concentrations of Bile Acids, we recommend spiking samples with a known amount of TBA Standard (0.072 nmol).

2. **Standard Curve Preparation:** Prepare 1 mM Bile Acids Standard by adding 10 µl of 100 mM TBA Standard to 990 µl of ddH_2O. Further dilute to 12 µM by adding 12 µl of 1 mM Bile Acids Standard to 988 µl ddh_2O. Add 0, 2, 4, 6, 8, and 10 µl of 12 µM TBA Standard into a series of wells in a 96-well plate to generate 0, 24, 48, 72, 96 and 120 pmol of Bile Acids/well. Adjust the volume to 50 µl/well with ddH_2O.
   Note:
   a. The assay measures Enzymatic Activity Rates (Abs/min). For maximum accuracy, we recommend to carry out a Standard Curve at the same time samples are measured.
3. **Probe Mix:** Dilute TBA Probe 50-fold (i.e. 2 µl TBA Probe + 98 µl TBA Probe Buffer). Mix enough reagents for the total number of wells to be assayed. Mix & add 100 µl of Probe mix/well to Standards, sample background & sample wells. Incubate for 10 min at 37 °C.
4. **Reaction Mix:** Prepare 50 µl Reaction Mix for each well to be assayed as below and mix well.

	Reaction Mix	*Background Control Mix
TBA Cycling Assay Buffer	46 µl	48 µl
TBA Cycling Enzyme Mix	2 µl	----
NADH	2 µl	2 µl

   Add 50 µl of Reaction Mix into Standard, and sample wells. Mix well. * For background correction, add Background Control Mix to sample background control well(s) and mix well.
5. **Measurement:** Measure absorbance at 405 nm in a kinetic mode at 37 °C for 60 min, protected from light. Choose two time points (T_1 & T_2) in the linear range to calculate the slope of every assayed well. Slopes for Standards, sample background control, and samples should be calculated using the same time points.
6. **Calculation:** Subtract 0 TBA Standard slope from all Standard readings. Plot the TBA Standard Curve. If sample background control slope is significant, then subtract sample background control slope from sample slope. Apply corrected slope to the Standard Curve to get B nmol TBA in the sample well.

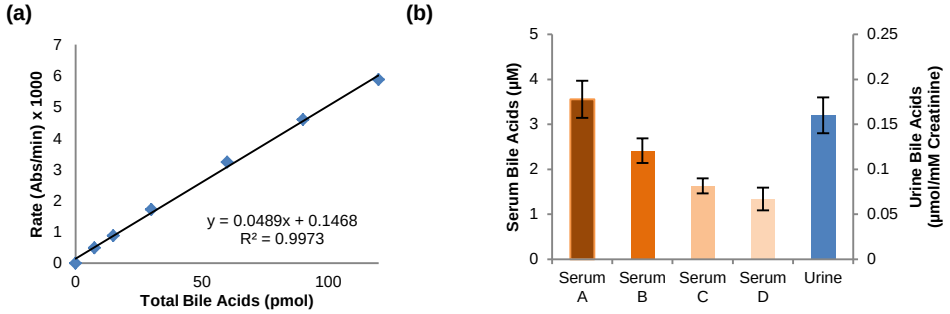
Sample TBA Concentration (C) = B/V X D nmol/µl or mM

Where: **B** is amount of TBA in the sample well from the Standard Curve (nmol)
V is sample volume added into the reaction well (µl)
D is sample dilution factor

Note: For spiked samples, correct for any sample interference by using the following equation:

$$\textbf{TBA amount in spiked sample wells (B)} = \frac{\mathrm{OD}_{\text{Sample slope (corrected)}}}{(\mathrm{OD}_{\text{Spiked sample slope (corrected)}}) - (\mathrm{TBA}_{\text{Sample slope (corrected)}})} \text{ x TBA spiked (nmol)}$$

1 mM ≡ 1000 µM
Bile Acids Molecular Weight: 521.69



Figures: (a) Total Bile Acids Standard Curve. **(b)** 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.

VIII. Related Products:

Alanine Aminotransferase (ALT or SGPT) Activity Colorimetric/Fluorometric Assay Kit (K752)
Aspartate Aminotransferase (AST or SGOT) Activity Colorimetric Assay Kit (K753)
Gamma Glutamyl Transferase (GGT) Activity Colorimetric Assay Kit (K784), Fluorometric Assay Kit (K785)
Adenosine Deaminase Activity Assay Kit (Colorimetric) (K321), (Fluorometric) (K328)
Adenosine Deaminase Activity Assay Kit Bilirubin (Total and Direct) Colorimetric Assay Kit (K553)
Sodium deoxycholate (2830)
Chenodeoxycholic acid (2831)
Lithocholic acid (2187)

FOR RESEARCH USE ONLY! Not to be used on humans.