

Version 4c, Last updated 19 June 2025

# **ab239702**

## **Total Bile Acids Assay Kit (Colorimetric)**

For the detection of Bile Acids in various biological samples.

This product is for research use only and is not intended for diagnostic use.

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## 1. Overview

Total Bile Acids Assay Kit (Colorimetric) (ab239702) provides a simple, sensitive, and high-throughput adaptable approach to detect physiological concentration of total bile acids in a variety of biological fluids including serum, plasma, bile, urine and saliva. 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 total bile acids in the sample. The assay can detect as little as 1  $\mu\text{M}$  of Bile Acids in a variety of samples.

## 2. Protocol Summary

Prepare samples and standards, add to wells and adjust volume to 50  $\mu\text{L}$  with ddH<sub>2</sub>O .



Add 100  $\mu\text{L}$  of Probe mix to the wells of the Standards, sample background & samples and incubate for 10 min at 37°C.



Add 50  $\mu\text{L}$  of Reaction Mix or background control mix to appropriate wells.



Measure absorbance at 405 nm in a kinetic mode at 37°C for 60 min, protected from light.

### **3. General guidelines, precautions, and troubleshooting**

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:  
[www.abcam.com/assaykitguidelines](http://www.abcam.com/assaykitguidelines)
- For typical data produced using the assay, please see the assay kit datasheet on our website.

#### 4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
TBA Cycling Assay Buffer	7 mL	-20°C
TBA Probe Buffer	14 mL	-20°C
TBA Probe	1 vial	-20°C
TBA Cycling Enzyme Mix	1 vial	-20°C
NADH I	1 vial	-20°C
TBA Standard	1 vial	-20°C

#### 5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

## 6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

### 6.1 TBA Cycling Assay Buffer:

Store at -20°C or 4°C. Bring to room temperature (RT) before use.

### 6.2 TBA Probe Buffer:

Store at -20°C or 4°C. Bring to room temperature (RT) before use.

### 6.3 TBA Probe:

Reconstitute with 220 µL TBA Probe Buffer. Protect from light. Aliquot and store at -20°C. Bring to RT before use.

### 6.4 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.

### 6.5 NADH I:

Reconstitute with 220 µL of ddH<sub>2</sub>O. 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.

### 6.6 TBA Standard:

Reconstitute with 100 µL of ddH<sub>2</sub>O to generate 100 mM Bile Acids Standard. Dissolve completely. Store at -20°C. Use within 2 months.

## 7. Standard Preparation

- Always prepare a fresh set of standards for every use.
  - Discard working standard dilutions after use as they do not store well.
- 7.1** Prepare 1 mM Bile Acids Standard by adding 10  $\mu\text{L}$  of 100 mM TBA Standard to 990  $\mu\text{L}$  of ddH<sub>2</sub>O.
- 7.2** Further dilute to 12  $\mu\text{M}$  by adding 12  $\mu\text{L}$  of 1 mM Bile Acids Standard to 988  $\mu\text{L}$  ddH<sub>2</sub>O.
- 7.3** Add 0, 2, 4, 6, 8, and 10  $\mu\text{L}$  of 12  $\mu\text{M}$  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  $\mu\text{L}$ /well with ddH<sub>2</sub>O.

Standard #	12 $\mu\text{M}$ Bile Acids Standard ( $\mu\text{L}$ )	ddH <sub>2</sub> O ( $\mu\text{L}$ )	Bile Acids/well
1	10	40	120 pmol
2	8	42	96 pmol
3	6	44	72 pmol
4	4	46	48 pmol
5	2	48	24 pmol
6	0	50	0 pmol

**Δ Note:** 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.

## 8. Sample Preparation

- 8.1 Serum and urine samples can be assayed directly.
- 8.2 Add 5-50  $\mu\text{L}$  undiluted sample to a 96-well plate.
- 8.3 Adjust the volume to 50  $\mu\text{L}$ /well with ddH<sub>2</sub>O.

### **Δ Note:**

- Bile Acids concentrations vary over a wide range depending on the sample. TBA range concentrations in some biological samples are:
  - human serum: < 10  $\mu\text{M}$
  - human urine (adult): 0-30  $\mu\text{mol}/\text{mmol}$
  - Creatinine; Saliva: 0-5  $\mu\text{M}$ .
- For unknown samples, we recommend doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range.
- 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  $\mu\text{l}$ /well ddH<sub>2</sub>O.
- 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).

## 9. Assay Procedure

- 9.1** Dilute TBA Probe 50-fold (i.e. 2  $\mu\text{L}$  TBA Probe + 98  $\mu\text{L}$  TBA Probe Buffer). Mix enough reagents for the total number of wells to be assayed.
- 9.2** Mix & add 100  $\mu\text{L}$  of Probe mix to the wells of the Standards, sample background & samples added at Steps 7 and 8. Incubate for 10 min at 37°C.
- 9.3** Prepare 50  $\mu\text{L}$  Reaction Mix for each well to be assayed as per the table and mix well. Add 50  $\mu\text{L}$  of Reaction Mix into Standard, and sample wells. Mix well.

	<b>Reaction Mix</b>	<b>Background Control Mix</b>
TBA Cycling Assay Buffer	46 $\mu\text{L}$	48 $\mu\text{L}$
TBA Cycling Enzyme Mix	2 $\mu\text{L}$	-
NADH I	2 $\mu\text{L}$	2 $\mu\text{L}$

**Δ Note:** For background correction, add Background Control Mix to background control well(s) and mix well.

- 9.4** Measure absorbance at 405 nm in a kinetic mode at 37°C for 60 min, protected from light. Choose two time points (T1 and T2) in the linear range to calculate the slope of every assayed well. Slopes for Standards, backgrounds, and samples should be calculated using same time points.

## 10. Data Analysis

- 10.1** Subtract 0 TBA Standard slope from all Standard readings. Plot the TBA Standard Curve.
- 10.2** If sample background control slope is significant, then subtract sample background control slope from sample slope. Apply corrected reading to Standard Curve to get x nmol TBA in the sample well.

$$\text{Sample TBA Concentration (C)} = \text{B/V} \times \text{D nmol}/\mu\text{L or mM}$$

Where: **B** is amount of TBA in the sample well from Standard Curve (nmol)

**V** is sample volume added into the reaction well ( $\mu\text{L}$ )

**D** is sample dilution factor

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

TBA amount in spiked sample wells (B) =

$$\frac{\text{OD}_{\text{sample slope (corrected)}}}{(\text{OD}_{\text{spiked sample slope(corrected)}}) - (\text{OD}_{\text{sample slope (corrected)}})} \times \text{TBA spike (nmol)}$$

**Δ Note:**

1 mM  $\equiv$  1000  $\mu\text{M}$

Bile Acids Molecular Weight: 521.69

## 11. Typical Data

Typical data provided for demonstration purposes only.

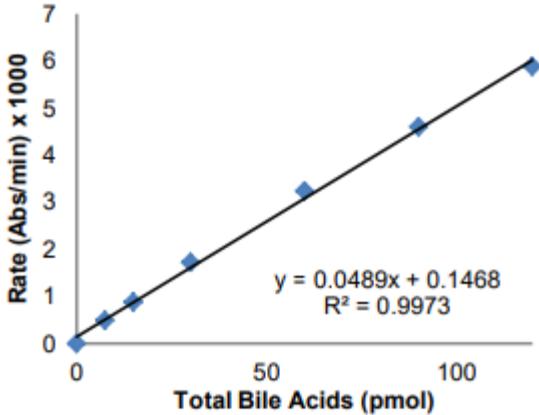


Figure 1. Total Bile Acids Standard Curve.

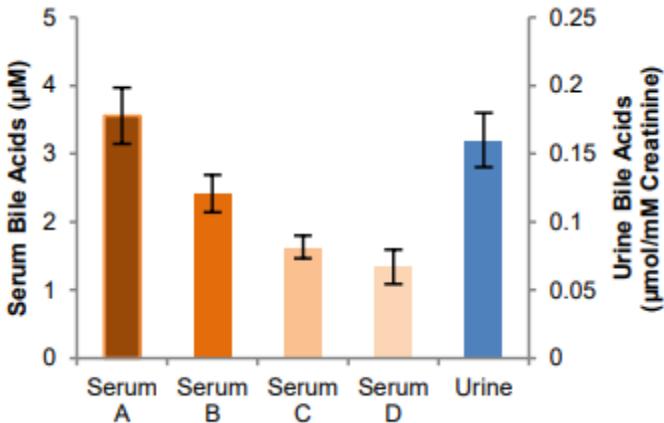


Figure 2. Estimation of Bile Acids concentration in human serum and urine. 30  $\mu\text{L}$  of each undiluted sample was assayed following the kit protocol. Bile Acids concentrations are: Serum (in  $\mu\text{M}$ ): A:  $3.56 \pm 0.41$ , B:  $2.41 \pm 0.27$ , C:  $1.63 \pm 0.17$ , D:  $1.34 \pm 0.25$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

## 13. Notes





## **Technical Support**

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