

## Instruction - Total Bile Acid Microplate Assay Kit

Catalog # ARS6761 (Version 1.4A)

Detection and Quantification of Total Bile Acid(TBA) Content in Urine, Serum, Tissue extracts, Other biological fluids Samples.

*For research use only. Not for diagnostic or therapeutic procedures.*

## 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 but 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.

Total Bile Acid Colorimetric Microplate Assay Kit provides a convenient colorimetric method to measure total bile acids in biological samples. In the assay, 3 $\alpha$ -hydroxysteroid dehydrogenase reacts with all bile acids, the color intensity at 405 nm is linear to the bile acid concentration in the sample.

**II. KIT COMPONENTS**

<b>Component</b>	<b>Volume</b>	<b>Storage</b>
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Substrate Diluent	15 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Enzyme Diluent	4 ml x 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

### III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Hot air circulation oven

#### IV. REAGENT PREPARATION

**Substrate:** Add 15 ml Substrate Diluent to dissolve before use.

**Enzyme:** Add 4 ml Enzyme Diluent to dissolve before use.

**Standard:** Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2500  $\mu\text{mol/L}$  of standard stock/top solution, store at 4 °C for 1 month after reconstitution. Then dilute to 200  $\mu\text{mol/L}$  standard top solution by adding 0.08 ml stock solution into 0.92 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 200/100/50/25/12.5/6.25/3.125  $\mu\text{mol/L}$ .

## V. SAMPLE PREPARATION

### 1. For urine, serum, or other biological fluids samples

Detect directly.

### 2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

## VI. ASSAY PROCEDURE

Warm all reagents to 37°C before use.

Add following reagents into the microplate:

<b>Reagent*</b>	<b>Sample**</b>	<b>Standard</b>	<b>Blank</b>
Substrate	150 µl	150 µl	150 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Mix, incubate at 37°C for 5 minutes.			
Enzyme	40 µl	40 µl	40 µl
Mix, keep at 37°C for 5 minutes, record absorbance measured at 405 nm.			

Note: \*Reagents must be added sequentially and should not be premixed prior to addition. \*\*The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

## VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n \text{ (}\mu\text{mol / L)}$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the weight of sample

$$C = \frac{(C_{\text{standard}} \times V_{\text{Standard}}) \times (OD_{\text{sample}} - OD_{\text{Blank}})}{(OD_{\text{standard}} - OD_{\text{Blank}}) \times W \times (V_{\text{sample}} / V_{\text{Assay}})} \text{ (}\mu\text{mol / g)}$$

2.2 According to the volume of sample

$$C = \frac{(C_{\text{standard}} \times V_{\text{standard}}) \times (OD_{\text{sample}} - OD_{\text{Blank}})}{(OD_{\text{standard}} - OD_{\text{Blank}}) \times V_{\text{sample}}} \text{ (}\mu\text{mol / ml)}$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of sample, g

Standard: the standard concentration, mmol/L= $\mu$ mol/ml

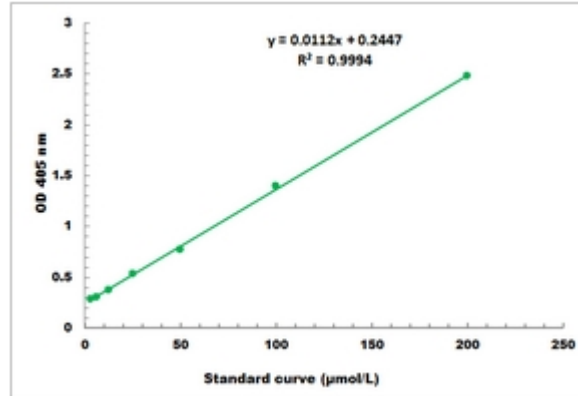
Standard: the volume of standard in assay procedure,  $\mu$ l

VSample: the volume of sample in assay procedure,  $\mu$ l

Assay: the volume of Assay Buffer,  $\mu$ l

### VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



$$y=0.0112x + 0.2447 \quad R^2=0.9994$$

Detection Range: 2 µmol/L - 200 µmol/L

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