Total Bile Acid

Microplate Assay Kit

User Manual

Catalog # CAK1166

(Version 1.3A)

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 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.

Total Bile Acid Microplate Assay Kit provides a convenient colorimetric method to measure total bile acids in biological samples. In the assay, 3α-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

Component	Volume	Storage
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
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Note:

Substrate: add 15 ml Substrate Diluent to dissolve before use.

Enzyme: add 4 ml Enzyme Diluent to dissolve before use.

Standard: add 1 ml Distilled water to dissolve before use, then add 0.08 ml into 0.92 ml Distilled water, mix. The concentration will be 200 µmol/L, store at 4 °C

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. 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.

V. ASSAY PROCEDURE

Warm all reagents to 37 °C before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
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:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

$$TBA\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$TBA\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

W: the weight of sample, g;

$C_{Standard}$: the protein concentration, 200 μmol/L = 0.2 μmol/ml;

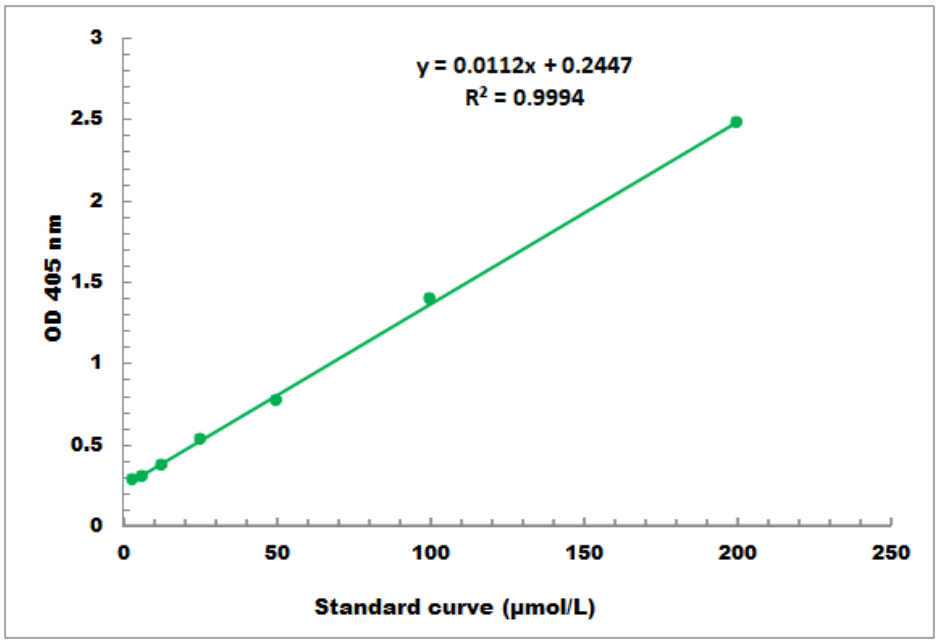
$V_{Standard}$: the volume of the standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay Buffer, 1 ml.

VII. TYPICAL DATA

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



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES