NOTE: Revision to
Assay Protocol

Product Manual

Total Bile Acid Assay Kit

Catalog Number

STA- 631 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Bile is a complex mixture of lipids, protein, carbohydrates, mineral salts, vitamins, and various trace elements, with bile acids making up about 67% of the total composition. Bile acids are produced from excess cholesterol, secreted from the liver, absorbed into the small intestines, and returned to the liver with portal blood. While bile acid synthesis is critical for the removal of cholesterol from the body, bile acids are also needed for proper uptake of dietary lipids, fat soluble vitamins, and other nutrients into the small intestines. Under physiological conditions, newly synthesized bile acids are conjugated to glycine or taurine to form bile salts, and not much free bile acid is actually found in bile.

Determining circulatory levels of bile acids can be used to identify or diagnose certain liver diseases. In addition, elevated serum bile levels have been observed in intrahepatic cholestasis of pregnancy cases.

Cell Biolabs' Total Bile Acid Assay Kit is a simple colorimetric assay that measures the amount of total bile acid present in plasma, serum, tissue homogenates, or cell lysates in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, bile acid standards and unknown samples. Sample bile acid concentrations are determined by comparison with a known bile acid standard.

Assay Principle

Cell Biolabs' Total Bile Acid Assay Kit measures the total bile acid within serum, plasma, and cell or tissue lysate samples. The assay is based on an enzyme driven reaction: when bile acids are incubated in the presence of 3α-hydroxysteroid dehydrogenase (3α-HSD), NADH, and thio-NAD^+, thio-NAD^+ is converted to its reduced form Thio-NADH. Thio-NADH is then detected colorimetrically as an absorbance increase at 405 nm (Figure 1).

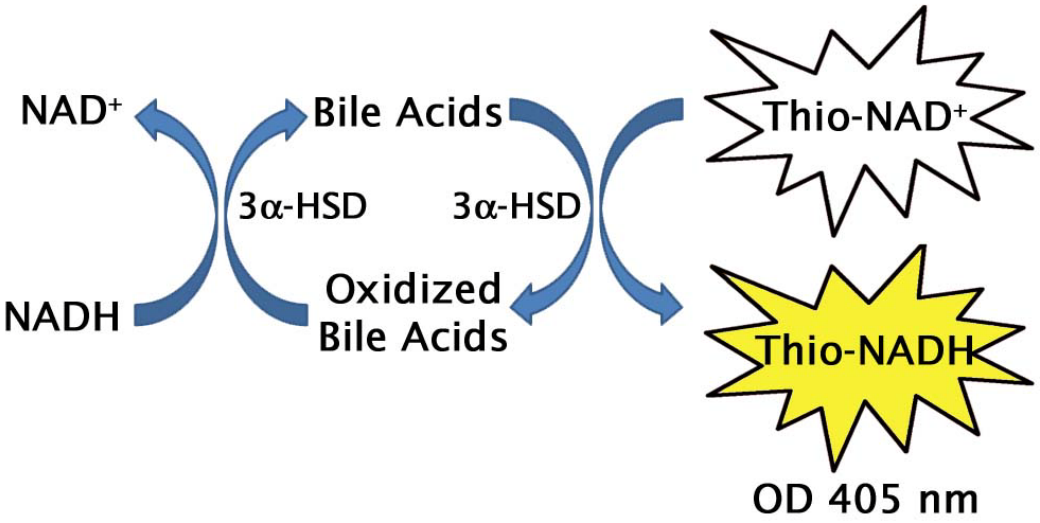

Figure 1. Total Bile Acid Assay Principle

Related Products

1. STA-241: Human Low Density Lipoprotein
2. STA-242: Human Very Low Density Lipoprotein
3. STA-243: Human High Density Lipoprotein
4. STA-361: Human ApoAI and ApoB Duplex ELISA Kit
5. STA-362: Human ApoAI ELISA Kit
6. STA-363: Human ApoAII ELISA Kit
7. STA-364: Human ApoCI ELISA Kit
8. STA-365: Human ApoCII ELISA Kit
9. STA-366: Human ApoCIII ELISA Kit
10. STA-367: Human ApoE ELISA Kit
11. STA-368: Human ApoB-100 ELISA Kit
12. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
13. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit

Kit Components

1. Bile Acid Standard (Part No. 263101): One 200 µL vial of a 250 µM glycochenodeoxycholic acid solution in water.
2. Assay Reagent A (Part No. 263102): One 30 mL amber bottle containing thio-NAD^+.
3. Assay Reagent B (Part No. 263103): Three 2 mL vials containing 3α-HSD and NADH.
4. NADH Reagent (Part No. 263104): Three 2 mL vials containing NADH.

Materials Not Supplied

1. 96 well plate or 96 well strips
2. Distilled or deionized water
3. 1X PBS
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate Reader

Storage

Upon receipt, store Assay Reagent A at -20ºC. Store all other components at -80ºC. If the kit will be used in multiple experiments, aliquot each component before freezing to avoid multiple freeze-thaw cycles.

Preparation of Samples

Samples should be assayed immediately or stored at -80ºC prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.

- Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Aliquot the supernatant for storage at -80ºC. Perform dilutions in deionized H_2O.
- Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Aliquot the supernatant for storage at -80ºC. Perform dilutions in deionized H_2O.
- Serum: Avoid hemolyzed and lipemic blood samples. Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in deionized H_2O as necessary.
- Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in deionized H_2O as necessary.

Preparation of Bile Acid Standard Curve

Prepare fresh bile acid standards by diluting in deionized H_2O according to Table 1 below.

Tubes	250 µM Bile Acid Standard (µL)	Deionized H_2O (µL)	Resulting Bile Acid Concentration (µM)
1	20	180	25
2	100 of Tube #1	100	12.5
3	100 of Tube #2	100	6.25
4	100 of Tube #3	100	3.12
5	100 of Tube #4	100	1.56
6	100 of Tube #5	100	0.78
7	100 of Tube #6	100	0.39
8	0	100	0

Table 1. Preparation of Bile Acid Standards.

Assay Protocol

Each Bile Acid standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Note: Each standard or sample replicate requires two paired wells, one to be treated with 3α-HSD *(Reagent B) and one without the enzyme (NADH).*

1. Add 20 µL of the diluted bile acid standards or samples to the 96-well microtiter plate.
2. Add 150 µL of Assay Reagent A to each well and mix contents thoroughly.
3. Incubate at 37ºC for 5 minutes.
4. Add 50 µL of NADH Reagent to one half of the paired standard or sample wells and mix the well contents thoroughly.
5. Add 50 µL of Assay Reagent B to the other half of the paired wells and mix thoroughly.
6. Incubate at room temperature for 30 minutes on an orbital shaker.
7. Read the plate at a primary wavelength of 405 nm and a secondary wavelength 630 nm using a microplate spectrophotometer.

Example of Results

The following figures demonstrate typical Total Bile Acid Assay results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

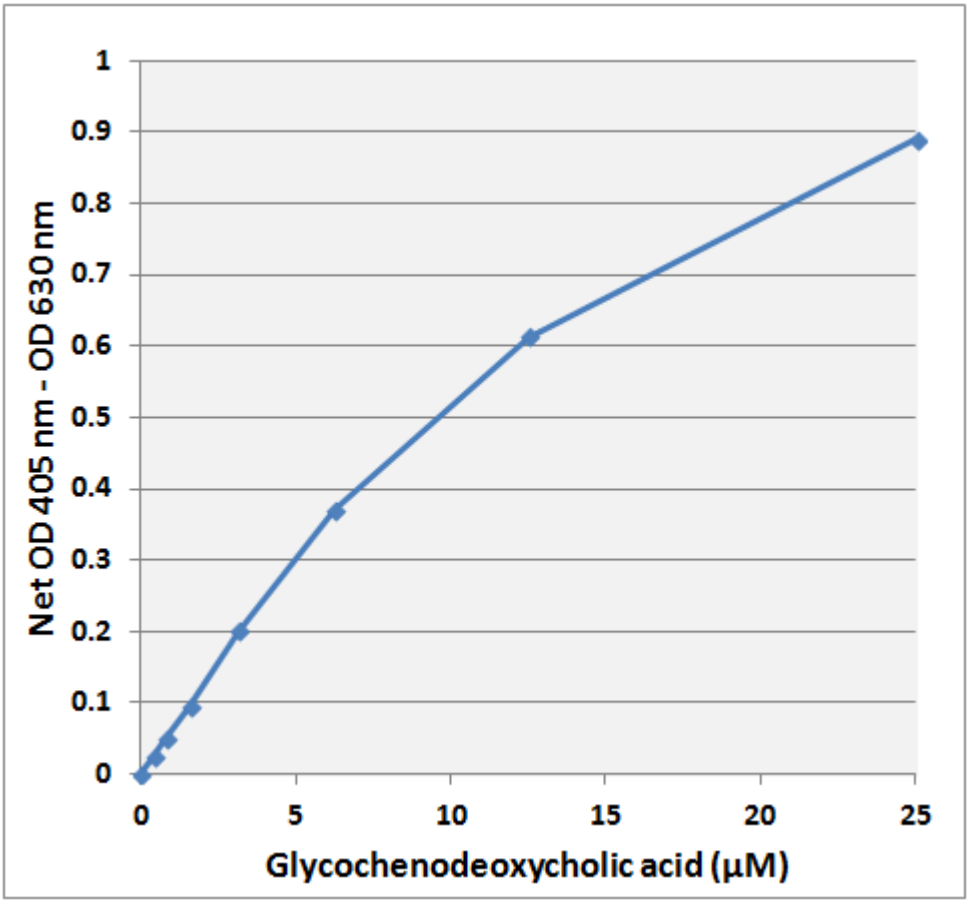

Figure 2: Bile Acid Standard Curve.

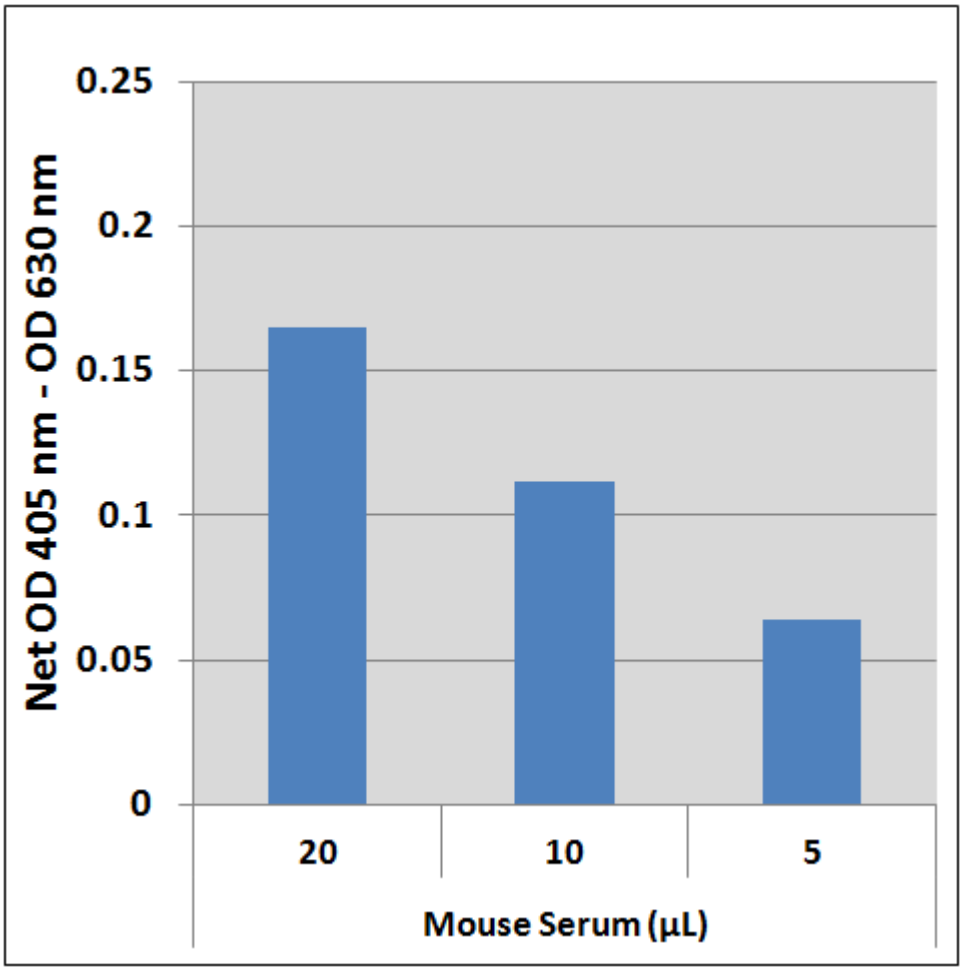

Figure 3: Mouse Serum tested with the Total Bile Acid Assay kit. Mouse serum samples were tested according to the Assay Protocol.

Calculation of Results

1. Subtract the 630 nm absorbance from the 405 nm absorbance.
2. Determine the average absorbance values for each sample, control, and standard.
3. Subtract the average zero standard value from itself and all standard values.
4. Graph the standard curve (see Figure 2).
5. Subtract the sample well values without 3α-HSD (NADH) from the sample well values containing enzyme (Assay Reagent B) to obtain the difference. The absorbance difference is due to the enzyme 3α-HSD activity:

$$\Delta A = A_{Rgt\ B} - A_{NADH}$$

6. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of bile acid present in the sample. Only use values within the range of the standard curve.

References

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Recent Product Citations

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