NOTE: Revision to
Assay Protocol

Product Manual

Total Bile Acid Assay Kit (Fluorometric)

Catalog Number

MET-5005 **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 fluorometric 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) and NAD^+, NAD^+ is converted to its reduced form NADH. Diaphorase then uses NADH to reduce resazurin to resorufin which is then detected fluorometrically (Figure 1).

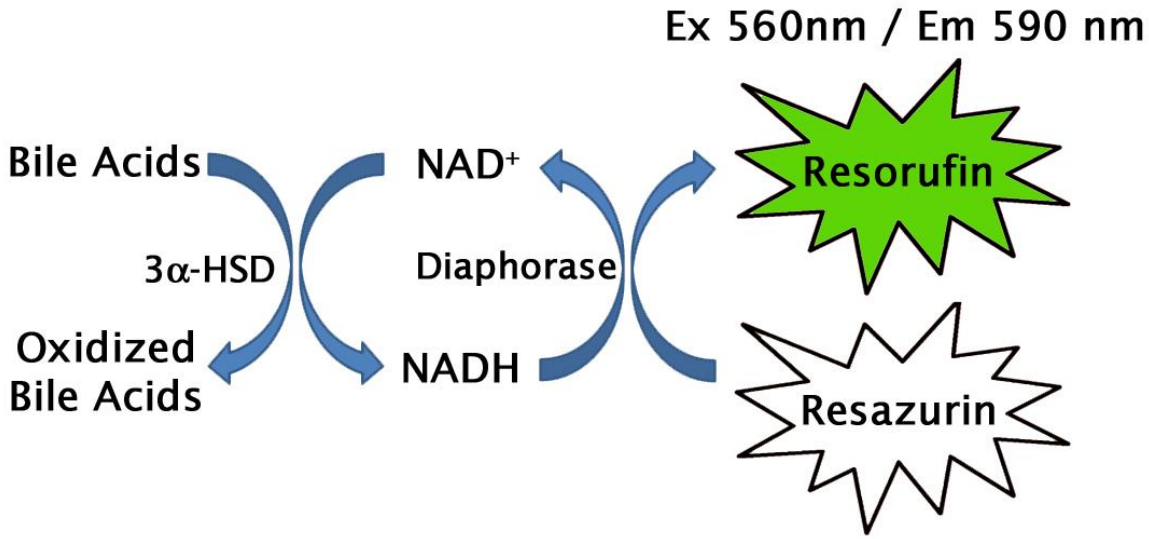

Figure 1. Total Bile Acid Assay Principle.

Related Products

1. STA-390: Total Cholesterol Assay Kit (Fluorometric)
2. MET-5055: L-Amino Acid Assay Kit (Fluorometric)
3. MET-5007: Cholic Acid Elisa Kit (Colorimetric)
4. MET-5008: Chenodeoxycholic Acid Elisa Kit
5. MET-5071: Taurine Assay Kit

Kit Components

1. Bile Acid Standard (Part No. 50051C): One 300 µL vial of a 250 µM glycochenodeoxycholic acid solution in water.
2. Assay Reagent (Part No. 50052D): Three 1.7 mL vials containing 3α-HSD, NAD+, diaphorase, and resazurin.
3. 5X Assay Buffer (Part No. 50053B): One 2 mL vial.
4. NAD+ Reagent (Part No. 50054D): Three 1.7 mL vials containing NAD+, diaphorase, and resazurin.

Materials Not Supplied

1. 96 well black plate
2. Distilled or deionized water
3. Microplate Fluorometer

Storage

Upon receipt, store the kit at -80ºC.

Preparation of Reagents

Note: 5X Assay Buffer must be brought to room temperature prior to use.

- 1X Assay Buffer: Dilute the stock 5X Assay Buffer 1:5 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store unused 1X Assay Buffer at 4°C.

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 isopropanol and centrifuge at 10,000 x g for 10 minutes at 4ºC. Aliquot the supernatant for storage at -80ºC. Samples must be diluted at least 5-10-fold in deionized H_2O prior to testing in the assay.

- Cell Lysates: Sonicate or homogenize cells in cold isopropanol and centrifuge at 10,000 x g for 10 minutes at 4ºC. Aliquot the supernatant for storage at -80ºC. Samples must be diluted at least 5-10-fold in deionized H_2O prior to testing in the assay.
- 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. Dilute samples at least 1:4 in deionized H_2O and perform further dilutions 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. Dilute samples at least 1:4 in deionized H_2O and perform further dilutions 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	30	270	25
2	150 of Tube #1	150	12.5
3	150 of Tube #2	150	6.25
4	150 of Tube #3	150	3.12
5	150 of Tube #4	150	1.56
6	150 of Tube #5	150	0.78
7	150 of Tube #6	150	0.39
8	0	150	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 sample replicate requires two paired wells, one to be treated with 3α-HSD *(Assay Reagent) and one without the enzyme (NAD+ Reagent).*

1. Add 50 µL of the diluted bile acid standards or samples to the 96-well microtiter black plate.
2. Add 50 µL of Assay Reagent to the standards and one half of the paired sample wells.
3. Add 50 µL of NAD+ Reagent to the other half of the paired sample wells.
4. Add 100 µL of 1X Assay buffer to all wells and mix the well contents thoroughly.
5. Incubate at room temperature for 45-60 minutes protected from light.
6. Read the plate at an excitation wavelength of 560 nm and an emission wavelength 590 nm using a microplate fluorometer.

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.

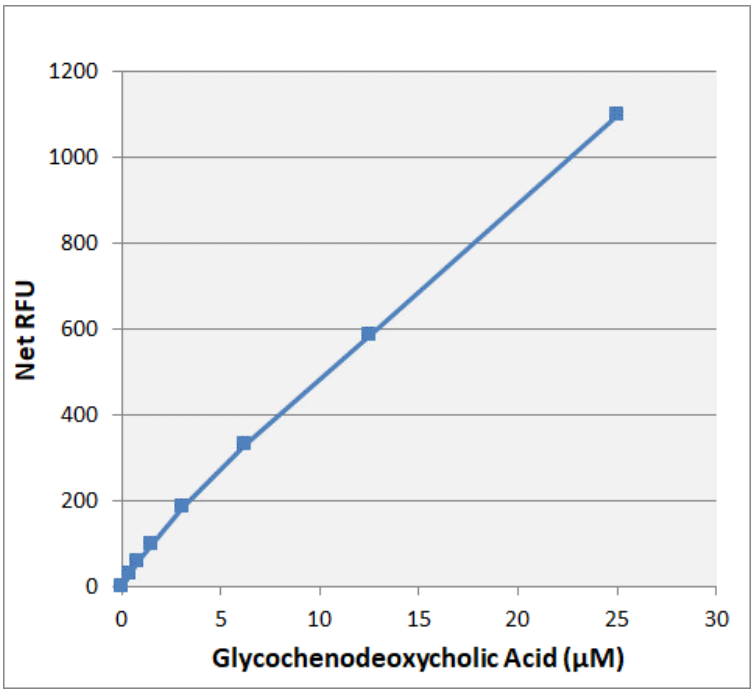

Figure 2: Bile Acid Standard Curve.

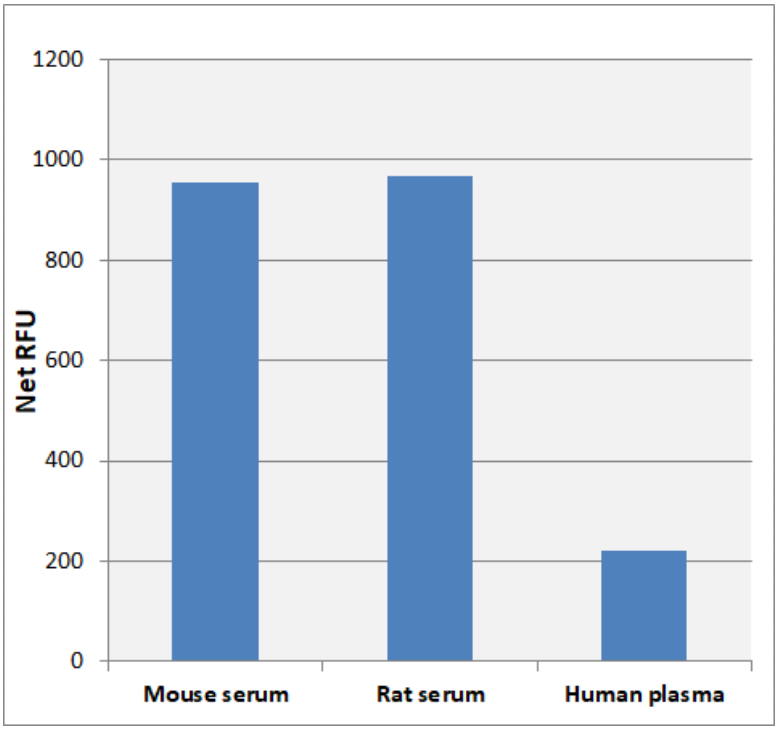

Figure 3: Bile Acid Content in Samples from Various Species. Serum or plasma samples were diluted 1:8 and then 50 µL samples were tested according to the Assay Protocol.

Calculation of Results

1. Determine the average Relative Fluorescence Unit (RFU) values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without 3αHSD (-3αHSD) from the sample well values containing 3αHSD (+3αHSD) to obtain the difference. The fluorescence difference is due to the 3αHSD activity.

   net RFU = (RFU+3αHSD) - (RFU-3αHSD)

5. Compare the net RFU of each sample to the standard curve to determine and extrapolate the quantity of bile acids present in the sample. Only use values within the range of the standard curve.

References

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3. Angelin B., Bjorkhem I., and Einarsson K. (1978) *J. Lipid Res*. **19**: 527-537.
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Recent Product Citations

1. Gwon, K. et al. (2023). Function of hepatocyte spheroids in bioactive microcapsules is enhanced by endogenous and exogenous hepatocyte growth factor. *Bioact Mater*. **28**:183-195. doi: 10.1016/j.bioactmat.2023.05.009.
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4. Lin, T. et al. (2019). Manipulation of the dry bean (Phaseolus vulgaris L.) matrix by hydrothermal and high-pressure treatments: Impact on in vitro bile salt-binding ability. *Food Chemistry*. doi: 10.1016/j.foodchem.2019.125699.
5. Meixiong, J. et al. (2019). MRGPRX4 is a G protein-coupled receptor activated by bile acids that may contribute to cholestatic pruritus. *Proc Natl Acad Sci U S A*. pii: 201903316. doi: 10.1073/pnas.1903316116.

Warranty

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