Product Manual

Total Bile Acid Assay Kit (Fluorometric)

Catalog Number
MET-5005 100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures
**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-631: Total Bile Acid Assay Kit (Colorimetric)
2. STA-361: Human ApoAI and ApoB Duplex ELISA Kit
3. STA-362: Human ApoAI ELISA Kit
4. STA-366: Human ApoCIII ELISA Kit
5. STA-367: Human ApoE ELISA Kit
6. STA-368: Human ApoB-100 ELISA Kit
7. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
8. STA-391: HDL and LDL/VLDL Cholesterol 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. 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 Fluorometer

Storage
Upon receipt, store the kit at -80°C.

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 -80°C 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 4°C. Aliquot the supernatant for storage at -80°C. Samples must be diluted at least 5-10-fold in deionized H₂O 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 4°C. Aliquot the supernatant for storage at -80°C. Samples must be diluted at least 5-10-fold in deionized H₂O 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 -80°C. Dilute samples at least 1:4 in deionized H₂O 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 4°C for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80°C. Dilute samples at least 1:4 in deionized H₂O and perform further dilutions as necessary.

**Preparation of Bile Acid Standard Curve**
Prepare fresh bile acid standards by diluting in deionized H₂O according to Table 1 below.

<table>
<thead>
<tr>
<th>Tubes</th>
<th>250 µM Bile Acid Standard (µL)</th>
<th>Deionized H₂O (µL)</th>
<th>Resulting Bile Acid Concentration (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>270</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
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<td>150</td>
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<tr>
<td>8</td>
<td>0</td>
<td>150</td>
<td>0</td>
</tr>
</tbody>
</table>

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.](image-url)
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.

\[
\text{net RFU} = (\text{RFU}_{+3\alpha\text{HSD}}) - (\text{RFU}_{-3\alpha\text{HSD}})
\]

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

Recent Product Citations

Warranty
These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS’s sole obligation and purchaser’s exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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