**Product Information**

**Bile Acid Assay Kit**

Catalog Number MAK309  
Storage Temperature –20 °C

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**TECHNICAL BULLETIN**

**Product Description**

Twelve different types of bile acids are typically found in mammals, among them two primary types are cholic acid and chenodeoxycholic acid. These can be dehydroxylated into secondary bile acids. Finally, these four can be conjugated to either taurine or glycine creating 8 different conjugated bile acids. Bile acid levels in feces, blood, urine, and bile can be used as markers for various diseases such as gall stones, hyperlipidemia, cholestasis, colon cancer, etc.

Bile acids also exist as sulfate salt forms known as bile acid sulfates. Sulfation of bile acids increases their solubility and decreases intestinal absorption, thereby enhancing fecal and urinary excretion. This assay does not measure bile acid sulfates, and measures only the twelve non-sulfated bile acids.

This Bile Acid Assay Kit provides a convenient fluorimetric means to measure total bile acids in biological samples. In the assay, 3α-hydroxysteroid dehydrogenase reacts with all twelve bile acids, converting NAD to NADH, which reduces a probe to a highly fluorescent product. The resulting fluorescence intensity (\( \lambda_{ex} = 530 \text{ nm} / \lambda_{em} = 585 \text{ nm} \)) is linear to the bile acid concentration in the sample.

Safe: Non-radioactive assay.

Sensitive and accurate: Linear detection range of 1–150 μM bile acids.

Convenient and high-throughput: Homogeneous “mix-incubate-measure” type assay. No wash and reagent transfer steps are involved. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

**Components**

The kit is sufficient for 100 assays in 96 well plates.

- **Assay Buffer**  
  Catalog Number MAK309A  
  10 mL

- **NAD Solution**  
  Catalog Number MAK309B  
  1 mL

- **Probe**  
  Catalog Number MAK309C  
  750 μL

- **Enzyme A**  
  Catalog Number MAK309D  
  120 μL

- **Enzyme B**  
  Catalog Number MAK309E  
  120 μL

- **Standard**  
  Catalog Number MAK309F  
  120 μL

**Reagents and Equipment Required but Not Provided.**

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.

**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**

The provided components are ready to use. Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

**Storage/Stability**

The kit is shipped on dry ice. Storage at –20 °C, is recommended.
**Procedure**

All samples and standards should be run in duplicate. Use ultrapure water for the preparation of Sample, Internal Standard, and Sample Blank.

Use black flat-bottom plates. Prior to assay, bring all reagents to room temperature. Briefly centrifuge enzyme tubes and keep on ice during assay. Urine samples can be stored at room temperature for 1-2 days, 4 °C for 6 days, and at −20 °C for 2 weeks. Serum samples can be stored at −20 °C for 3 weeks.

Three wells will be needed per sample: Sample, Internal Standard, and Sample Blank.

1. **Internal Standard**: Prepare 250 μL of 80 μM sodium cholate by mixing 20 μL of Standard and 230 μL of ultrapure water.
2. Transfer 20 μL of sample to each of the three wells.
3. Add 5 μL of ultrapure water to Sample and Sample Blank wells, and 5 μL of Internal Standard to the Internal Standard well.
4. **Working Reagent**: For Internal Standard and Sample wells, prepare Working Reagent for each well by mixing 75 μL of Assay Buffer, 8 μL of NAD, 4 μL of Probe, 1 μL of Enzyme A and 1 μL of Enzyme B.
   a. For the Sample Blank wells, prepare Blank Reagent for each well by mixing 75 μL of Assay Buffer, 8 μL of NAD, 4 μL of Probe and 1 μL of Enzyme B (i.e., NO Enzyme A).
   b. Add 80 μL of Working Reagent to Internal Standard and Sample wells, and 80 μL of Blank Reagent to the Sample Blank wells.
5. Tap plate to mix. Incubate for 20 minutes in the dark. Read fluorescence intensity (λ兴奋 = 530 nm/λ发射 = 585 nm).

**Results**

**Calculation**

Bile acid concentration of a Sample is calculated as:

\[
[Bile \text{ Acids}] = \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}} - 20 \times \text{ n}}{F_{\text{STANDARD}} - F_{\text{SAMPLE}}}
\]

Where:

- \(F_{\text{SAMPLE}}\), \(F_{\text{STANDARD}}\), and \(F_{\text{BLANK}}\) are the fluorescence intensity values of the Sample, Internal Standard, and Sample Blank wells, respectively.
- 20 μM is the effective concentration of the Internal Standard (Internal Standard volume is 1/4 the volume of the Sample).
- \(n\) is the dilution factor.

Note: If the Sample bile acid concentration is higher than 150 μM, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

**References**

## Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Suggested Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay not working</strong></td>
<td>Cold assay buffer</td>
<td>Assay Buffer must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Omission of step in procedure</td>
<td>Refer and follow Technical Bulletin precisely</td>
</tr>
<tr>
<td></td>
<td>Plate reader at incorrect wavelength</td>
<td>Check filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Type of 96 well plate used</td>
<td>For fluorometric assays, use black plates with clear bottoms</td>
</tr>
<tr>
<td><strong>Samples with erratic readings</strong></td>
<td>Samples prepared in different buffer</td>
<td>Use the Assay Buffer provided or refer to Technical Bulletin for instructions</td>
</tr>
<tr>
<td></td>
<td>Cell/Tissue culture samples were incompletely homogenized</td>
<td>Repeat the sample homogenization, increasing the length and extent of homogenization step</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple freeze-thaw cycles</td>
<td>Aliquot and freeze samples if samples will be used multiple times</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>If possible, dilute sample further</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples and store correctly until use</td>
</tr>
<tr>
<td><strong>Lower/higher readings in samples and standards</strong></td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>Use of expired kit or improperly stored reagents</td>
<td>Check the expiration date and store the components appropriately</td>
</tr>
<tr>
<td></td>
<td>Allowing the reagents to sit for extended times on ice</td>
<td>Prepare fresh Reaction Mix before use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
</tr>
<tr>
<td></td>
<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
</tr>
<tr>
<td><strong>Non-linear standard curve</strong></td>
<td>Use of partially thawed components</td>
<td>Thaw and resuspend all components before preparing the reaction mix</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors in preparation of standards</td>
<td>Avoid pipetting small volumes</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors in the Reaction Mix</td>
<td>Prepare a Reaction Mix whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the plate well</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Refer to the standard dilution instructions in the Technical Bulletin</td>
</tr>
<tr>
<td></td>
<td>Calculation errors</td>
<td>Recheck calculations after referring to Technical Bulletin</td>
</tr>
<tr>
<td></td>
<td>Substituting reagents from older kits/lots</td>
<td>Use fresh components from the same kit</td>
</tr>
<tr>
<td><strong>Unanticipated results</strong></td>
<td>Samples measured at incorrect wavelength</td>
<td>Check the equipment and filter settings</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>If possible, dilute sample further</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/below the linear range</td>
<td>Concentrate or dilute samples so readings are in the linear range</td>
</tr>
</tbody>
</table>