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Product Information

Bile Acid Assay Kit

Catalog Number **MAK309**
Storage Temperature –20 °C

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 (λ_{ex} = 530 nm/λ_{em} = 585 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.
- Fluorescence multiwell plate reader

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_{ex} = 530 nm/λ_{em} = 585 nm).

Results

Calculation

Bile acid concentration of a Sample is calculated as:

$$[\text{Bile Acids}]\ (\mu M) = \frac{F_{SAMPLE} - F_{BLANK}}{F_{STANDARD} - F_{SAMPLE}} \times 20 \times n$$

Where:

F_{SAMPLE}, $F_{STANDARD}$, and F_{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

1. Hanson, N.Q. et al., Effect of Protein on the Determination of Total Bile Acids in Serum. Clin. Chem., **29**(1), 171-175 (1983).
2. Makino, I. et al., Sulfated and Non-sulfated bile acids in urine, serum, and bile of patients with hepatobiliary diseases. Gastroenterology, **68**, 545-553 (1975).
3. Takafumi, K., Enzymatic Determination of Serum 3α-sulfated Bile Acids Concentration with Bile Acid 3α-sulfate Sulfohydrolase. Digestive Diseases and Sciences, **41**(8), 1564-1570 (1996).

Troubleshooting Guide

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

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