Bile Acid Assay Kit (Fluorometric)

Bile Acid Assay Kit (Fluorometric) is a detection kit for the quantification of Bile Acid in serum, plasma and urine.

Catalog number: ARG82140

Package: 100 tests

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INTRODUCTION

Bile acids are steroid acids found predominantly in the bile of mammals and other vertebrates. Diverse bile acids are synthesized in the liver. Bile acids are conjugated with taurine or glycine residues to give anions called bile salts.

Primary bile acids are those synthesized by the liver. Secondary bile acids result from bacterial actions in the colon. In humans, taurocholic acid and glycocholic acid (derivatives of cholic acid) and taurochenodeoxycholic acid and glycochenodeoxycholic acid (derivatives of chenodeoxycholic acid) are the major bile salts. They are roughly equal in concentration. The salts of their 7-alpha-dehydroxylated derivatives, deoxycholic acid and lithocholic acid, are also found, with derivatives of cholic, chenodeoxycholic and deoxycholic acids accounting for over 90% of human biliary bile acids.

Bile acids comprise about 80% of the organic compounds in bile (others are phospholipids and cholesterol). An increased secretion of bile acids produces an increase in bile flow. Bile acids facilitate digestion of dietary fats and oils. They serve as micelle-forming surfactants, which encapsulate nutrients, facilitating their absorption. These micelles are suspended in the chyme before further processing. Bile acids also have hormonal actions throughout the body, particularly through the farnesoid X receptor and GPBAR1 (also known as TGR5). [Provide by Wikipedia: Bile acid]

PRINCIPLE OF THE ASSAY

This Bile Acid Assay Kit (Fluorometric) is a simple fluorometric assay that measures the amount of bile acid present in serum, plasma and urine. 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 (λexc/em = 530/585 nm) is linear to the bile acid concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C. Shelf life of 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
NAD Solution	1 mL	-20°C
Probe	750 µL	-20°C
Enzyme A	120 µL	-20°C
Enzyme B	120 µL	-20°C
Standard	120 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm.
- Centrifuge
- Black flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- No wash and reagent transfer steps are involved. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: 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 at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Urine: To remove insoluble particles, centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant should be assayed directly.

Note:

1. 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.
2. 3 wells will be needed per sample: Sample, Internal Standard, and Sample Blank.

REAGENT PREPARATION

- **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.
- **Blank Reagent:** 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 (NO Enzyme A).
- **Internal Standard:** Prepare 250 µL of 80 µM sodium cholate by mixing 20 µL of standard and 230 µL of distilled water.

ASSAY PROCEDURE

Prior to the assay, equilibrate all components to room temperature. Briefly centrifuge enzyme tubes, keep on ice during assay. **3 wells will be needed per sample: Sample, Internal Standard, and Sample Blank.**

	Sample well	Standard well	Sample Blank well
Internal Standard		5 µL	
Samples	20 µL	20 µL	20 µL
Distilled water	5 µL		5 µL
Working Reagent	80 µL	80 µL	
Blank Reagent			80 µL
Tap microplate to mix well. Incubate for **20 minutes** in the dark.			
Read the plate with a fluorescence microplate reader using **excitation 530 nm filter** and **emission 585 nm filter**.			

CALCULATION OF RESULTS

1. Bile acid concentration of a sample is calculated as follow,

   Bile Acid (µM) = $[(RFU_{Sample} – RFU_{Blank}) / (RFU_{Standard} - RFU_{Sample})] \times 20 \times n$

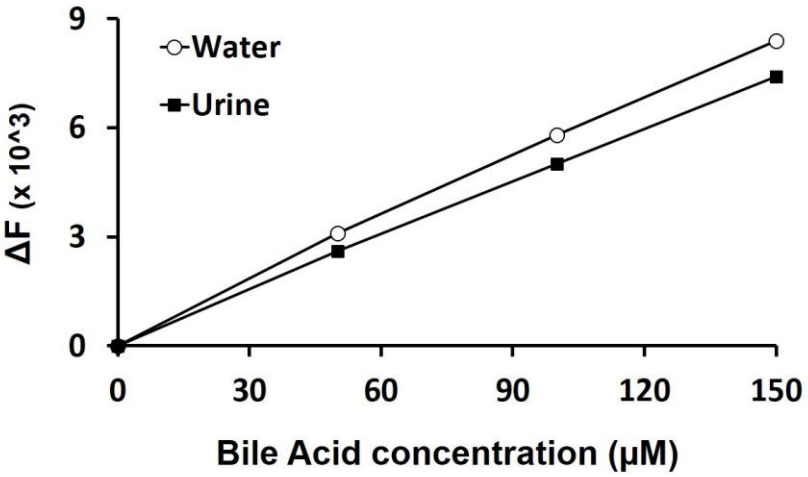
   Note:

   - RFU_{Sample}, $RFU_{Standard}$ and RFU_{Blank}: the fluorescence values of the Sample, Internal Standard and Sample Blank wells.
   - 20: the effective concentration of the Internal Standard (Internal Standard volume is ¼ the volume of the Sample).
   - n: the sample dilution factor.

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Bile Acid Assay Kit (Fluorometric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

1 µM