Datasheet for ABIN5067551

Total Bile Acid Assay Kit (Fluorometric)

3 Images

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Overview

Quantity:	100 tests
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	Total Bile Acid Assay Kit measures the total bile acid within serum, plasma, and cell or tissue lysate samples.
Detection Method:	Fluorometric
Characteristics:	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.
Components:	1. Bile Acid Standard : One 300 µL vial of a 250 M glycochenodeoxycholic acid solution in water. 2. Assay Reagent : Three 2 mL vials containing 3α-HSD, NAD+, diaphorase, and resazurin. 3. 5X Assay Buffer : One 2 mL vial.

Target Details

Background:	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,

Target Details

	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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Protocol:	The assay is based on an enzyme driven reaction: when bile acids are incubated α (3α + +in the presence of 3 -hydroxysteroid dehydrogenase -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 .
Reagent Preparation:	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.
Sample Preparation:	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 PBS and centrifuge at 10,000 x g for 10 minutes at 4 °C. Aliquot the supernatant for storage at -80 °C. Perform dilutions in deionized H2O. Cell lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4 °C. Aliquot the supernatant for storage at -80 °C. Perform dilutions in deionized H2O. 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.
Assay Procedure:	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.

Application Details

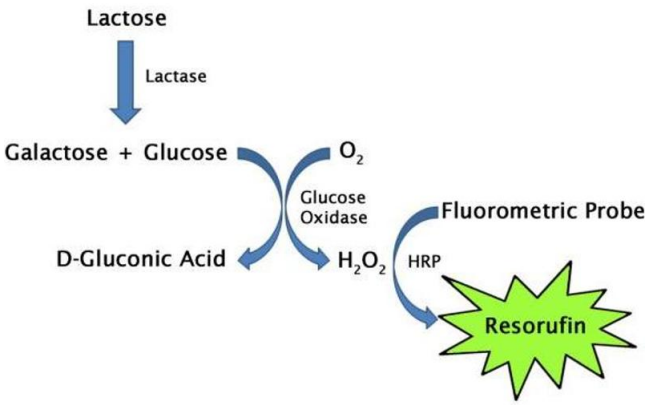
1. Add 50 µL of the diluted bile acid standards or samples to the 96-well microtiter black plate.
2. Add 50 µL of Assay Reagent to each well
3. Add 100 µL of 1X Assay buffer and mix the well contents thoroughly.
4. Incubate at room temperature for 45-60 minutes protected from light.
5. Read the plate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

Restrictions:	For Research Use only

Handling

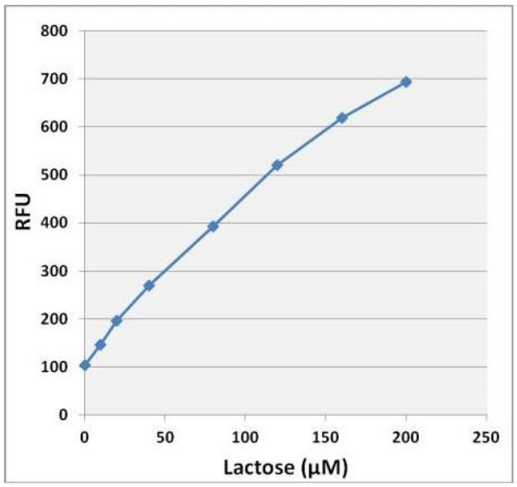
Storage:	-80 °C
Storage Comment:	Upon receipt, store the kit at -80°C.

Images



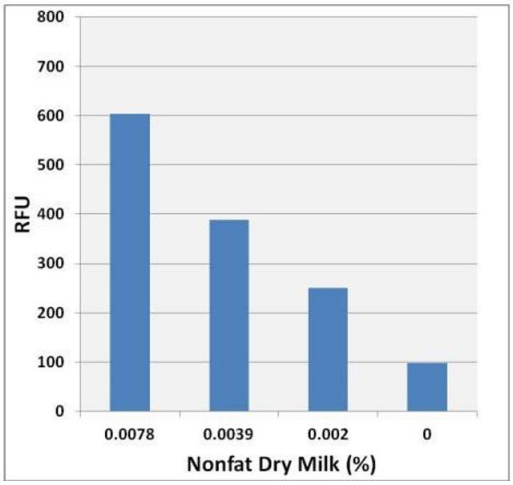
Biochemical Assay

Image 1. Total Bile Acid Assay Principle



Biochemical Assay

Image 2. Bile Acid Standard Curve



Biochemical Assay

Image 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.