Bile Acid Assay Kit (Fluorometric)

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

Catalog number: ARG82225

Package: 100 assays

For research use only. Not for use in diagnostic procedures.

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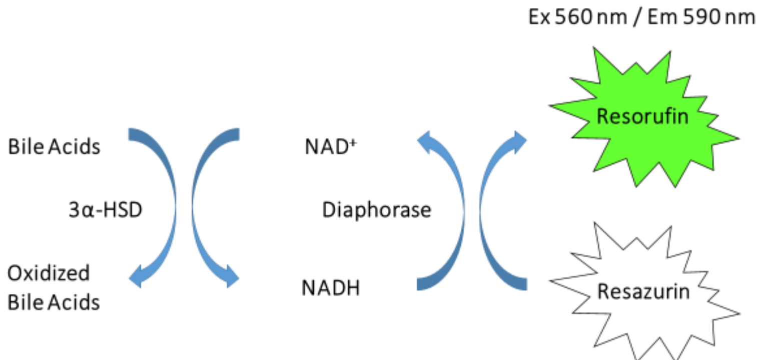
INTRODUCTION

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. [Provide by Wikipedia: Bile Acid]

PRINCIPLE OF THE ASSAY

This Bile Acid Assay Kit (fluorometric) employs a fluorogenic substrate to measure total bile acid in 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. (See Picture below) The RFU value is measured at an excitation wavelength of 560 nm and an emission wavelength 590 nm. The concentration of bile acid in the samples is then determined by comparing the RFU of samples to the standard curve.



MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at -80°C. Avoid multiple freeze/thaw cycles. Use the kit before expiration date.

Component	Quantity	Storage information
Bile Acid Standards (250 μM)	300 μL	-80°C
Assay Reagent (3α-HSD, NAD+, diaphorase, and resazurin)	1.7 mL	-80°C
5X Assay Buffer	2 mL	-80°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader equipped with a 560 nm excitation filter and 590 nm emission filter
- Deionized or Distilled water
- 96-well fluorescence black plate
- 1X PBS
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at -80°C at all times.
- 5X Assay Buffer must be brought to room temperature prior to use.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- 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.

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 water.

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 water.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Sample should be tested immediately or frozen at -80°C for storage. Dilute samples at least 1:4 in deionized water and perform further dilutions as necessary.

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. Sample should be tested immediately or frozen at -80°C for storage. Dilute samples at least 1:4 in deionized water and perform further dilutions as necessary.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum / plasma sample.

REAGENT PREPARATION

- **1X Assay Buffer:** Dilute 5X Assay Buffer into deionized water to yield 1X Assay Buffer. (E.g., add 1 mL of 5X Assay Buffer into 4 mL of deionized water to a final volume of 5 mL) Stir or vortex to homogeneity. Store 1X Assay Buffer at 4°C.
- **Standards:** Prepare fresh bile acid standards by diluting in deionized water according to the Table below.

Standard tube	Final Bile Acid conc. (µM)	Volume of Deionized Water (µL)	Volume of Bile Acid Standard (µL)
S1	25	270	30 of 250 µM Bile Acid Standard
S2	12.5	150	150 of S1
S3	6.25	150	150 of S2
S4	3.12	150	150 of S3
S5	1.56	150	150 of S4
S6	0.78	150	150 of S5
S7	0.39	150	150 of S6
S0	0	150	0

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

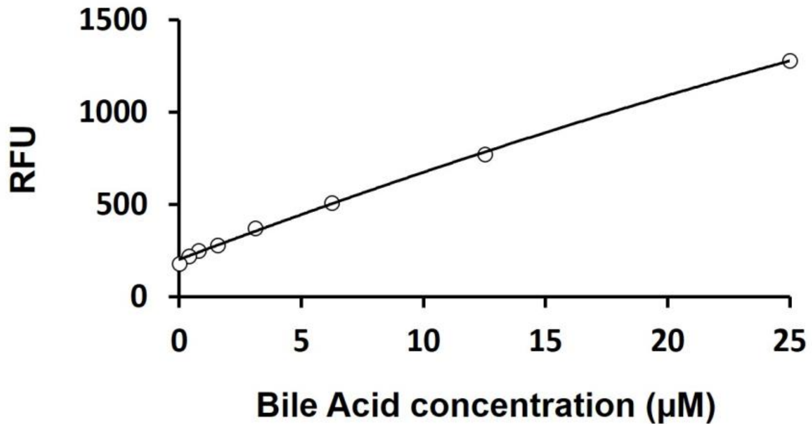
1. Add **50 µL** of **samples** or serial **diluted Bile Acid Standards** into 96-well fluorescence black plate.
2. Add **50 µL** of **Assay Reagent** into the appropriate wells of 96-well fluorescence black plate.
3. Add **100 µL** of **1X Assay Buffer** each well. Mix the well contents thoroughly.
4. Incubate for **45-60 minutes** at **room temperature**.
5. Read the plate at an **excitation wavelength** of **560 nm** and an **emission wavelength** of **590 nm** using a microplate fluorometer.

CALCULATION OF RESULTS

1. Calculate the average RFU for each set of standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean RFU obtained from each standard against its concentration with RFU on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean RFU for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit or linear curve fit.

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

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay was ≤ 10 %