Bile Acid Assay Kit (Colorimetric)

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

Catalog number: ARG82226

Package: 100 assays

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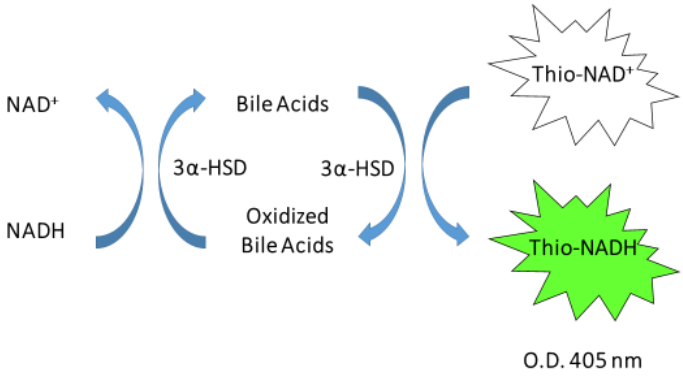
INTRODUCTION

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 (Colorimetric) employs a 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), NADH, and thio-NAD+, thio-NAD+ is converted to its reduced form Thio-NADH. Thio-NADH is then detected colorimetrically as an absorbance increase at 405 nm. The concentration of bile acid in the samples is then determined by comparing the 405 nm absorbance of samples to the standard curve.



MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store Assay Reagent A at -20°C. Store all other components at -80°C. If the kit will be used in multiple experiments, aliquot each component before freezing to avoid multiple freeze-thaw cycles.

Component	Quantity	Storage information
Bile Acid Standards (250 µM)	200 µL	-80°C
Assay Reagent A (Thio-NAD^+)	30 mL	-20°C
Assay Reagent B (3α-HSD and NADH)	3 X 1.7 mL	-80°C
NADH Reagent	3 X 1.7 mL	-80°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate Reader
- Deionized or Distilled water
- 96-well plate or strip
- 1X PBS
- 37°C incubator
- 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.
- Upon receipt, store Assay Reagent A at -20°C. Store all other components at -80°C. If the kit will be used in multiple experiments, aliquot each component before freezing to avoid multiple freeze-thaw cycles.
- Each sample replicate requires two paired wells, one to be treated with 3α-HSD (Reagent B) and one without the enzyme (NADH).
- 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

- **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	180	20 of 250 µM Bile Acid Standard
S2	12.5	100	100 of S1
S3	6.25	100	100 of S2
S4	3.12	100	100 of S3
S5	1.56	100	100 of S4
S6	0.78	100	100 of S5
S0	0	100	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.

Note: Each sample replicate requires two paired wells, one to be treated with 3α-HSD (Reagent B) and one without the enzyme (NADH).

1. Add **20 µL** of **samples** or serial **diluted Bile Acid Standards** into 96-well microplate.
2. Add **150 µL** of **Assay Reagent A** into the appropriate wells of 96-well microplate.
3. Incubate for **5 minutes** at **37°C**.
4. Add **50 µL** of **Assay Reagent B** to the standards and one half of the paired sample wells and mix thoroughly.
5. Add **50 µL** of **NADH Reagent** to other half of the paired sample wells and mix thoroughly.
6. Incubate for **30-60 minutes** at **37°C**.
7. Read the plate at a **primary wavelength of 405 nm** and a **secondary wavelength of 630 nm** using a microplate reader.

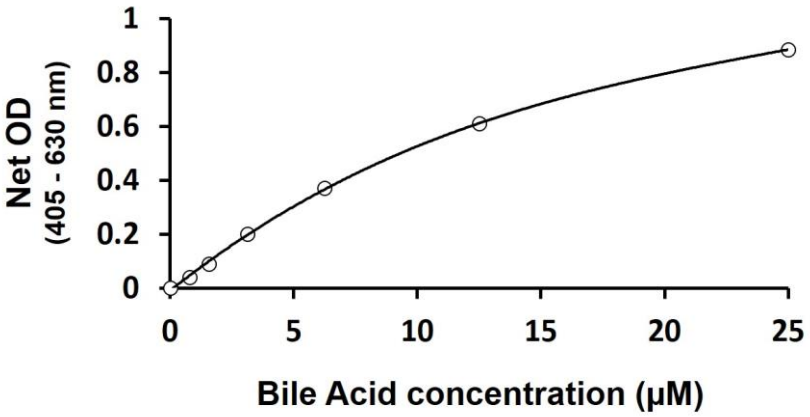
CALCULATION OF RESULTS

1. Subtract the value of 630 nm absorbance from the value of 405 nm absorbance.
2. Calculate the average absorbance value for each set of standards and samples.
3. Subtract the average value of Standard 0 from all standard value.
4. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
5. Subtract the sample well values without 3α-HSD (NADH) from the sample well values containing enzyme (Assay Reagent B) to obtain the difference. The absorbance difference is due to the enzyme 3α-HSD activity:

   $\Delta A = A_{\text{Reagent B}} - A_{\text{NADH}}$
6. Compare the change in absorbance Δ A of each sample to the standard curve to determine and extrapolate the quantity of bile acid present in the sample. Only use values within the range of the standard curve.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Bile Acid Assay Kit (Colorimetric). 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 %