Product Manual

Chenodeoxycholic Acid ELISA Kit

Catalog Number

MET-5008 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Chenodeoxycholic acid is a primary bile acid. Along with cholic acid, chenodeoxycholic acid is one of the two major bile acids synthesized from cholesterol by the liver. 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, 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. Recently, bile acids have been recognized as integrators of metabolic processes as well as signaling molecules.

The Chenodeoxycholic Acid ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of chenodeoxycholic acid in serum, feces, or other cell or tissue samples. The quantity of chenodeoxycholic acid in unknown samples is determined by comparing its absorbance with that of a known chenodeoxycholic acid standard curve. The kit has a detection sensitivity limit of 30 nM chenodeoxycholic acid. Each Chenodeoxycholic Acid ELISA Kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Assay Principle

The Chenodeoxycholic Acid ELISA kit is a competitive ELISA for the quantitative measurement of chenodeoxycholic acid. The unknown chenodeoxycholic acid samples or chenodeoxycholic acid standards are first added to a Chenodeoxycholic Acid Conjugate preadsorbed microplate. After a brief incubation, an anti-Chenodeoxycholic Acid monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The chenodeoxycholic acid content in unknown samples is determined by comparison with predetermined chenodeoxycholic acid standard curve.

Note: This kit detects chenodeoxycholic acid and glycochenodeoxycholic acid equally (See Figure 2).

Related Products

1. MET-5007: Cholic Acid ELISA Kit
2. MET-5005: Total Bile Acid Assay Kit (Fluorometric)
3. STA-631: Total Bile Acid Assay Kit (Colorimetric)
4. STA-361: Human ApoAI and ApoB Duplex ELISA Kit
5. STA-362: Human ApoAI ELISA Kit
6. STA-363: Human ApoAII ELISA Kit
7. STA-364: Human ApoCI ELISA Kit

8. STA-365: Human ApoCII ELISA Kit
9. STA-366: Human ApoCIII ELISA Kit
10. STA-367: Human ApoE ELISA Kit
11. STA-368: Human ApoB-100 ELISA Kit
12. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-Chenodeoxycholic Acid Antibody (500X) (Part No. 50081C): One 10 µL vial.
3. Secondary Antibody, HRP Conjugate (Part No. 230003): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.
8. Chenodeoxycholic Acid Standard (Part No. 50082B): One 100 µL vial of 2 mM Chenodeoxycholic Acid in water.

Box 2 (shipped on blue ice packs)

1. 100X Chenodeoxycholic Acid Conjugate (Part No. 50083C): One 100 µL vial.

Materials Not Supplied

1. Chenodeoxycholic acid samples such as serum, plasma, feces, or extracted from cells or tissues
2. Tissue/feces homogenizer
3. 1X PBS
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store both the Anti-Chenodeoxycholic Acid Antibody and 100X Chenodeoxycholic Acid Conjugate at -20ºC and avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- Chenodeoxycholic Acid Conjugate Coated Plate: Dilute the proper amount of 100X Chenodeoxycholic Acid Conjugate 1:100 into 1X PBS. Add 100 µL of the diluted 1X Chenodeoxycholic Acid Conjugate to each well and incubate at 37ºC for two hours or overnight at 4ºC. Remove the coating solution and wash twice with 200 µL of 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4ºC and remove the Assay Diluent immediately before use.

  Note: The Chenodeoxycholic Acid-Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Chenodeoxycholic Acid Antibody and Secondary Antibody: Immediately before use dilute the Anti-Chenodeoxycholic Acid Antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare Chenodeoxycholic Acid standards by diluting in 1X Assay Diluent. First, dilute the stock Chenodeoxcholic Acid Standard 2 mM solution 1:10 in 1X Assay Diluent for a 200 µM solution. (e.g. add 10 µL of the stock 2 mM standard to 90 µL of 1X Assay Diluent). Vortex thoroughly.
2. Use this 200 µM solution to prepare a series of the remaining standards according to Table 1 below.

Standard Tubes	200 µM Chenodeoxycholic Acid Standard (µL)	Assay Diluent (µL)	Chenodeoxycholic Acid (nM)
1	10	990	2000
2	500 of Tube #1	500	1000
3	500 of Tube #2	500	500
4	500 of Tube #3	500	250
5	500 of Tube #4	500	125
6	500 of Tube #5	500	62.5
7	500 of Tube #6	500	31.25
8	0	500	0

Table 1. Preparation of Chenodeoxcholic Acid Standards.

Preparation of Samples

- 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 -80ºC. Perform dilutions in Assay Diluent as necessary.

- Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in Assay Diluent as necessary.
- Cells, tissues, or feces: Homogenize 50-200 mg of the cell pellet, tissue, or feces in 0.5-2 mL of ice cold PBS using a mortar and pestle or by dounce homogenization. Incubate the homogenate at 4°C for 20 minutes. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Recover the supernatant and transfer to a fresh tube. Store resuspended sample at -20°C or colder until ready to test by ELISA. Perform dilutions in Assay Diluent as necessary.

Assay Protocol

Note: If testing mouse or rat plasma or serum, the IgG must be completely removed from each sample prior to testing, such as with Protein A or G beads. Additionally, a control well without primary antibody should be run for each sample to determine background signal.

1. Prepare and mix all reagents thoroughly before use. Each chenodeoxycholic acid sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or Chenodeoxycholic Acid standards to the wells of the Chenodeoxycholic Acid Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted Anti-Chenodoexycholic Acid antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody-HRP Enzyme Conjugate to all wells.
6. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash microwell strips 3 times according to step 4 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Chenodeoxycholic Acid ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

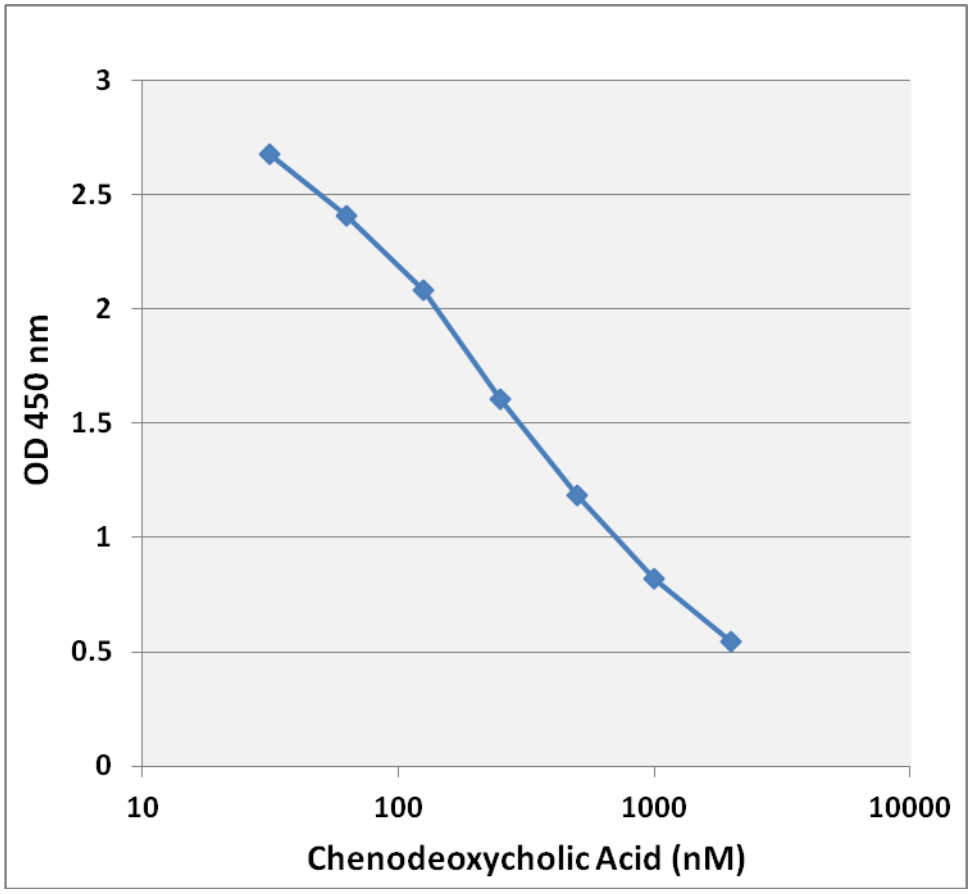

Figure 1: Chenodeoxycholic Acid ELISA Standard Curve.

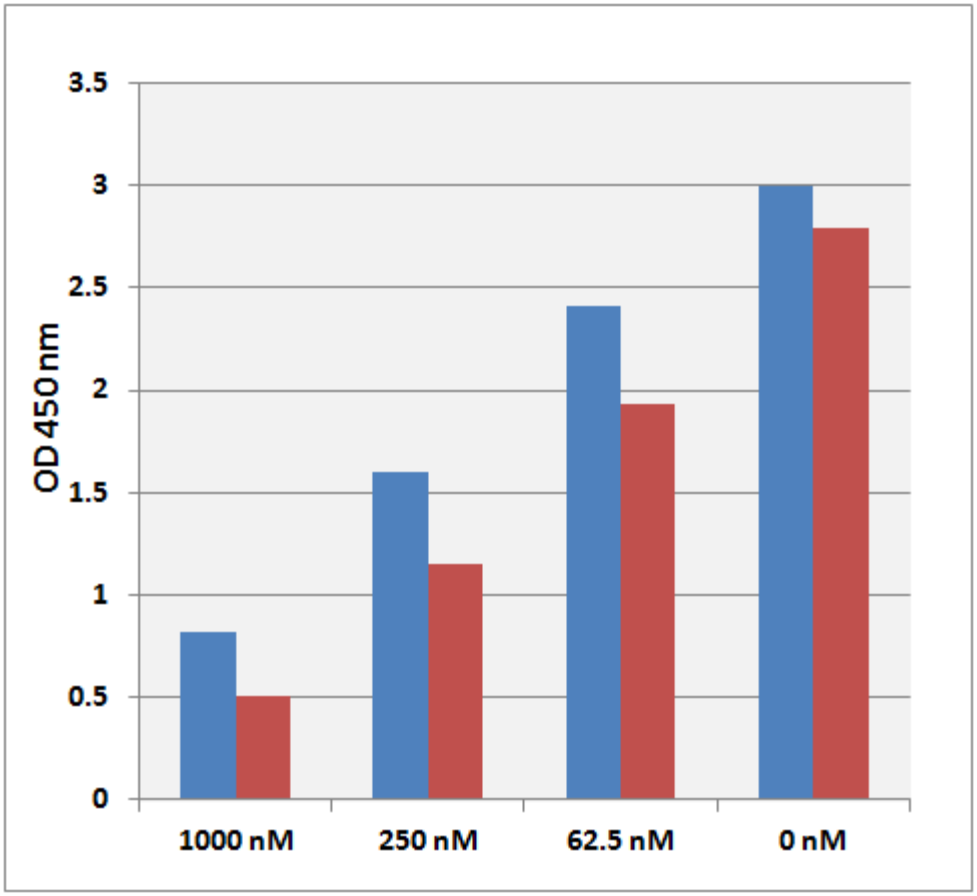

Figure 2: Comparison of Chenodeoxycholic Acid with Glycochenodeoxycholic Acid. Various dilutions of sodium chenodeoxycholic acid (blue bars) or sodium glycochenodeoxycholate (red bars) were tested according to the Assay Protocol.

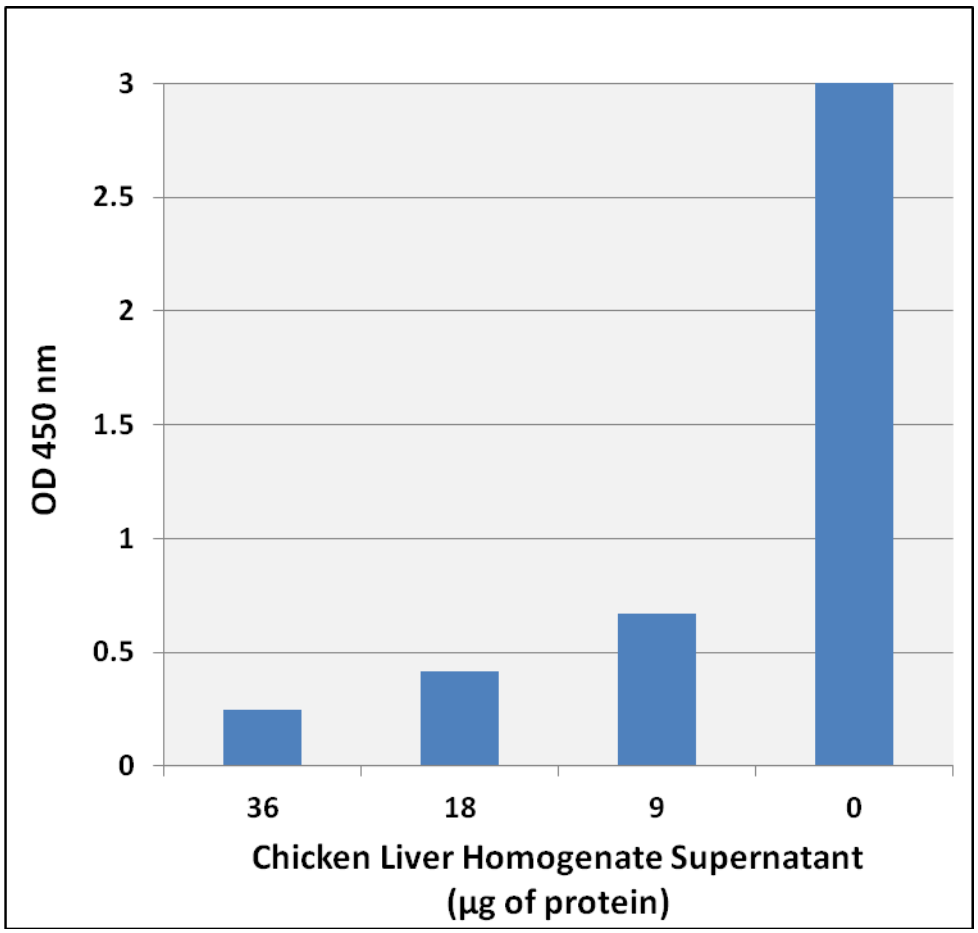

Figure 3: Chenodeoxyholic Acid Levels in Liver. Chicken liver was homogenized, and the homogenate supernatant (11.4 mg/mL protein by BCA assay) was diluted in Assay Diluent and analyzed according to the Assay Protocol.

Antibody Cross Reactivity

Chenodeoxycholic acid	100 %
Glycochenodeoxycholic acid	100 %
Cholesterol	0 %
Deoxycholic acid	0.6 %
Cholic acid	4 %

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Recent Product Citations

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3. Kong, X. et al. (2021). FXR-mediated epigenetic regulation of GLP-1R expression contributes to enhanced incretin effect in diabetes after RYGB. *J Cell Mol Med*. doi: 10.1111/jcmm.1633.
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