Revised Preparation of Standard Curve

Product Manual

# Cholic Acid ELISA Kit

**Catalog Number**

**MET-5007** **96 assays**

**FOR RESEARCH USE ONLY**
**Not for use in diagnostic procedures**

CELL BIOLABS, INC.
*Creating Solutions for Life Science Research*

## Introduction

Cholic acid is a primary bile acid. Along with chenodeoxycholic acid, cholic 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 Cholic Acid ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of cholic acid in serum, feces, or other cell or tissue samples. The quantity of cholic acid in unknown samples is determined by comparing its absorbance with that of a known cholic acid standard curve. The kit has detection sensitivity limit of 0.4 µM cholic acid. Each Cholic Acid ELISA Kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

## Assay Principle

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

## Related Products

1. MET-5005: Total Bile Acid Assay Kit (Fluorometric)
2. STA-631: Total Bile Acid Assay Kit (Colorimetric)
3. MET-5008: Chenodeoxycholic Acid Elisa Kit (Colorimetric)
4. MET-5071: Taurine Assay Kit (Colorimetric)
5. MET-5070: Glycine Assay Kit (Fluorometric)

## 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-Cholic Acid Antibody (500X) (Part No. 50071C): 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. Cholic Acid Standard (Part No. 50072B): One 100 µL vial of 2.5 mM Cholic Acid in 50 mM $KH_2PO_4$, pH 7.4, 25 mM NaCl.

**Box 2 (shipped on blue ice packs)**

1. 100X Cholic Acid Conjugate (Part No. 50073C): One 100 µL vial.

## Materials Not Supplied

1. Cholic acid samples such as serum, plasma, feces, or cholic acid extracted from cells or tissues
2. Tissue/Feces Homogenizer
3. 1X PBS

## Storage

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

## Preparation of Reagents

- Cholic Acid Conjugate Coated Plate: Dilute the proper amount of 100X Cholic Acid Conjugate 1:100 into 1X PBS. Add 100 µL of the diluted 1X Cholic 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 Cholic 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-Cholic Acid Antibody and Secondary Antibody: Immediately before use dilute the Anti-Cholic Acid Antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

## Preparation of Standard Curve

Use the provided stock Cholic Acid Standard 2.5 mM solution to prepare a series of the remaining standards according to Table 1.

| Standard Tubes | 2.5 mM Cholic Acid Standard (µL) | Assay Diluent (µL) | Cholic Acid (µM) |
|---|---|---|---|
| 1 | 10 | 990 | 25 |
| 2 | 500 of Tube #1 | 500 | 12.5 |
| 3 | 500 of Tube #2 | 500 | 6.25 |
| 4 | 500 of Tube #3 | 500 | 3.13 |
| 5 | 500 of Tube #4 | 500 | 1.56 |
| 6 | 500 of Tube #5 | 500 | 0.78 |
| 7 | 500 of Tube #6 | 500 | 0.39 |
| 8 | 0 | 500 | 0 |

**Table 1. Preparation of Cholic 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 cholic acid sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or Cholic Acid standards to the wells of the Cholic Acid Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted Anti-Cholic 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 Cholic Acid ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

**Cross Reactivity of Antibody**

| | |
|---|---|
| Cholic acid | 100 % |
| Cholesterol | 0 % |
| Deoxycholic acid | 5.1 % |
| Chenodeoxycholic acid | 11.9 % |
| Glycochenodeoxycholic acid | 8.0 % |

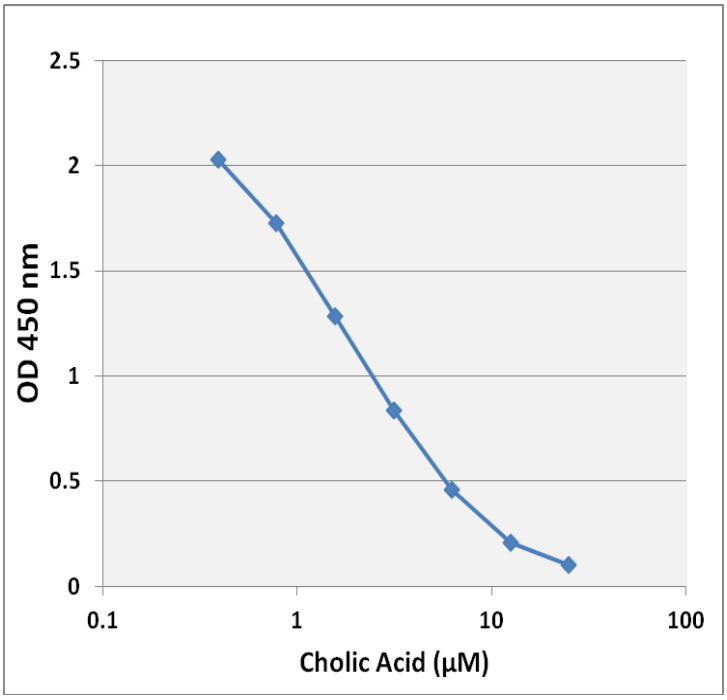


**Figure 1: Cholic Acid ELISA Standard Curve.**

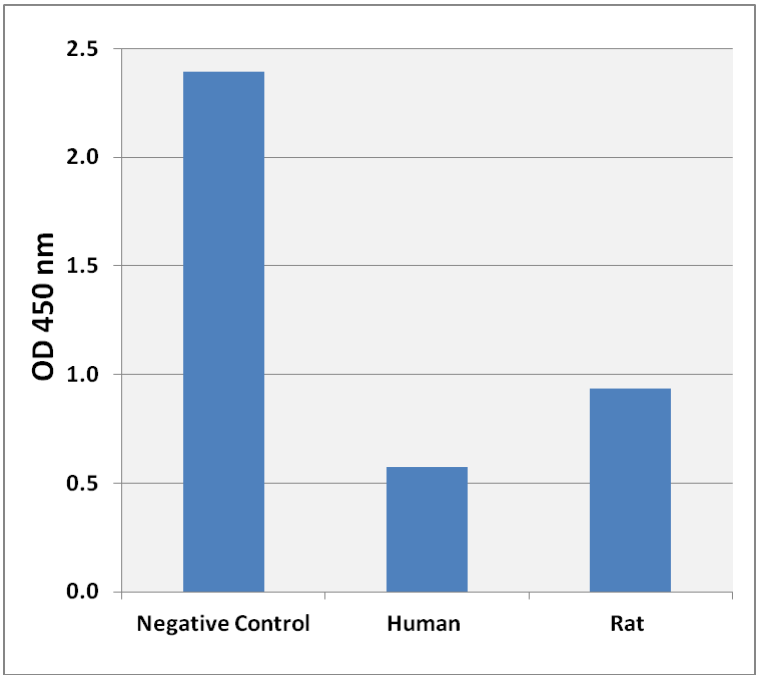


**Figure 2: Cholic Acid Levels in undiluted Human or Rat Serum compared to Negative Control (Assay Diluent).**

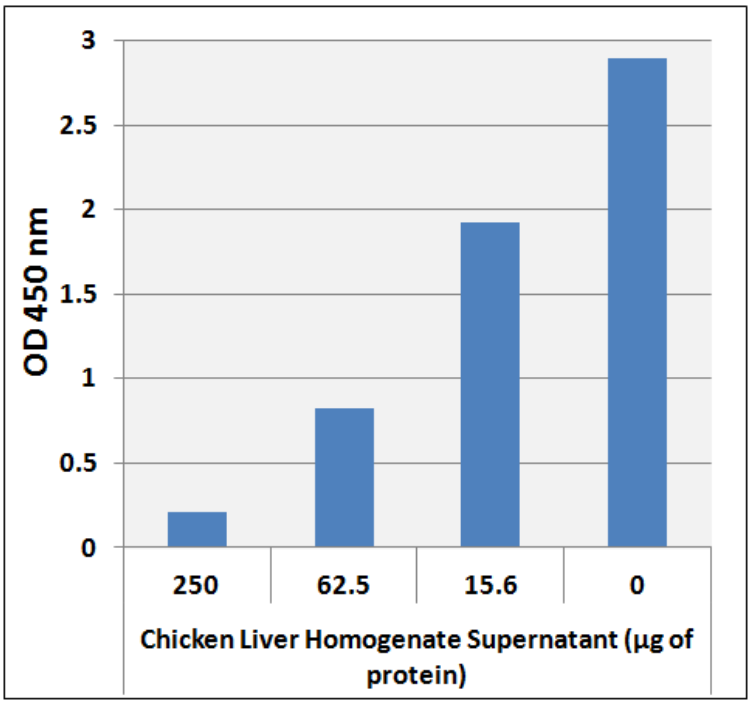


**Figure 3: Cholic Acid Levels in Liver.** Chicken liver was homogenized, and the homogenate supernatant (11.4 mg/mL protein by BCA assay) was diluted in Assay Diluent and analyzed according to the Assay Protocol.

## References


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## Recent Product Citations


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## Warranty

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