Cholic Acid ELISA Kit

Enzyme Immunoassay for the quantification of Cholic Acid in plasma, serum, feces, cell and tissue lysates samples.

Catalog number: ARG81202

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INTRODUCTION

Cholic acid, also known as 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid is a primary bile acid that is insoluble in water (soluble in alcohol and acetic acid), it is a white crystalline substance. Salts of cholic acid are called cholates. Cholic acid, along with chenodeoxycholic acid, is one of the two major bile acids produced by the liver, where it is synthesized from cholesterol. These two major bile acids are roughly equal in concentration in humans. Derivatives are made from cholyl-CoA, which exchanges its CoA with either glycine, or taurine, yielding glycocholic and taurocholic acid, respectively.

Cholic acid downregulates cholesterol-7-α-hydroxylase (rate-limiting step in bile acid synthesis), and cholesterol does the opposite. This is why chenodeoxycholic acid, and not cholic acid, can be used to treat gallstones (because decreasing bile acid synthesis would supersaturate the stones even more).

Cholic acid and chenodeoxycholic acid are the most important human bile acids. Other species may synthesize different bile acids as their predominant primary bile acids. [Provide by Wikipedia: Cholic acid]

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. Cholic Acid has been pre-coated onto a microtiter plate. Samples and standards are add in the plate. After a brief incubation, an Anti-Cholic Acid antibody is added into the plate. Cholic Acid in samples or standards and the Cholic Acid coated on wells are competing for a limited amount of Anti-Cholic Acid antibody. After incubation, the wells are washed with wash buffer to remove unbound material. Then an HRP conjugated secondary antibody is added into the plate. After incubation, the wells are washed to remove unbound material. The substrate solution (TMB substrate) is added and incubated, stopped with STOP solution, resulting in the development of a distinct color which absorbance is measured at 450 nm. The concentration of Cholic Acid in samples or standards is indirectly proportional to the color intensity or OD readings by the spectrometer.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, the Anti-Cholic Acid Antibody and 100X Cholic Acid should be aliquoted and stored at -20°C and avoid repeated freeze/thaw cycles. Store all other components at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
Protein binding microplate	8 X 12 strips	4°C
100X Cholic Acid	100 µL	–20°C
Standards (2.5 mM Cholic Acid)	100 µL	4°C
Diluent buffer	50 ml (ready to use)	4°C
Anti-Cholic Acid antibody (500X)	10 µL	–20°C
HRP Conjugated secondary antibody	20 µL	4°C
10X Washing Buffer	100 ml	4°C
TMB substrate	12 ml (ready to use)	4°C (protect from light)
STOP solution	12 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Tissue/Feces Homogenizer (optional)
- 1X PBS
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, the Anti-Cholic Acid Antibody and 100X Cholic Acid should be aliquoted and stored at-20°C and avoid repeated freeze/thaw cycles. Store all other components at 4°C. Use the kit before expiration date.
- If mouse or rat plasma or serum sample is used, the IgG in the samples should be completely removed by Protein A or G beads. Additionally, a sample control well with the same sample but without primary antibody should be included for background signal determination.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not

become completely dry during the assay.

- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

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.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

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.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Aliquot samples for testing and store at -80°C. Avoid repeated freeze-thaw cycles. Perform dilutions in Diluent buffer as necessary.
4. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash Buffer**: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g., add 50 ml of 10X Wash buffer into 450 ml of distilled water to a final volume of 500 ml) The 1X Washing Buffer is stable for up to 4 weeks at 2-8°C.
- **Anti-Cholic Acid antibody**: Immediately before use dilute the Anti-Cholic Acid antibody 1:500 with Diluent buffer.
- **HRP Conjugated secondary antibody**: Immediately before use dilute the HRP Conjugated secondary antibody 1:1000 with Diluent buffer.
- **Standard**: Prepare a series dilution of Cholic Acid standards with Diluent buffer. The Diluent buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with Diluent buffer as according to the suggested concentration table below:

Standard tubes	Cholic Acid (µM)	Diluent buffer (µL)	Cholic Acid standard (µL)
S1	25	990	10 of 2.5 mM stock standard
S2	12.5	500	500 of S1
S3	6.25	500	500 of S2
S4	3.13	500	500 of S3
S5	1.56	500	500 of S4
S6	0.78	500	500 of S5
S7	0.39	500	500 of S6
S0	0	500	0

Note: Dilutions for the standard must be made and applied to the plate immediately. S0 serves as background.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

Cholic Acid Plate Coating:

1. Dilute the proper amount of 100X Cholic Acid 1:100 into 1X PBS.
2. Add 100 µL of the diluted 1X Cholic Acid to each well and incubate at 37°C for two hours or overnight at 4°C.
3. Remove the coating solution and wash twice with 200 µL of 1X PBS.
4. Blot plate on paper towels to remove excess fluid.
5. Add 200 µL of Diluent buffer to each well and block for 1 hr at room temperature.
6. Transfer the plate to 4°C and remove the Diluent buffer immediately before use.

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

ELISA assay procedure:

1. Add 50 µL of sample or standards to the wells of the Cholic Acid -coated microplate. Incubate at room temperature for 10 minutes on a microplate shaker.

   Note: If mouse or rat plasma or serum sample is used. Additionally, a sample control well with the same sample but without primary antibody should be included for background signal determination.
2. Add 50 µL of the diluted Anti-Cholic Acid antibody to each well, incubate at room temperature for 1 hour on a microplate shaker.
3. Aspirate each well and wash, repeating the process 2 times for a total 3

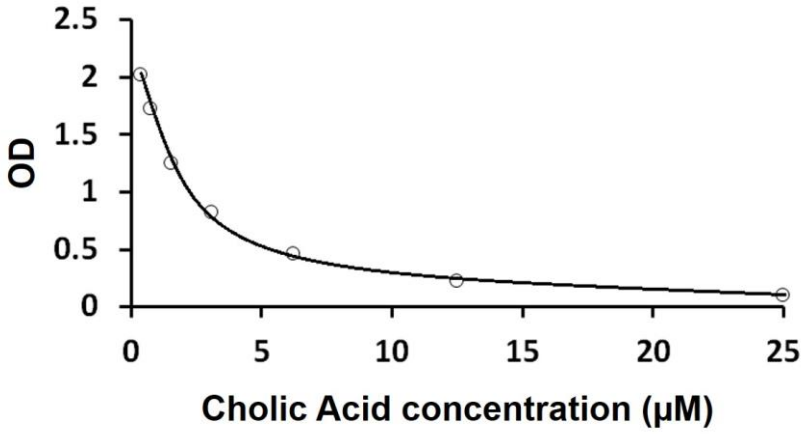
washes. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

4. Add **100 µL** of the diluted **HRP Conjugate secondary antibody** to all wells. Incubate at **room temperature** for **1 hour** on a microplate shaker.
5. Aspirate each well and **wash as step 3**.
6. Warm **TMB Substrate** to **room temperature**. Add **100 µl** of **TMB Substrate** to each well, including the blank wells. Incubate for **2-30 minutes** at room temperature in dark.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
7. Add **100 µl** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at **450nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 30 minutes** after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD CURVE

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.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value 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. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

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

Sensitivity

0.4 µM

Cross Reactivity of Antibody

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