

HUMAN CHOLESIN /C7ORF50 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
HUMAN CHOLESIN CONCENTRATIONS IN SERUM
AND PLASMA



ALWAYS REFER TO LOT SPECIFIC
PROTOCOL PROVIDED WITH EACH KIT FOR
INSTRUCTIONS. PROTOCOL MUST BE
READ AND CHECK ALL ITEMS OF EACH KIT
BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN
DIAGNOSTIC PROCEDURES.

**PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.**

ELISA NAME	HUMAN CHOLESIN ELISA KIT
Catalog No.	SK00027-06
Lot No.	
Formulation	96 T
Standard range	31.25 - 2000 pg/mL
Sensitivity	5 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma,
Dilution Factor	<i>Optimal dilutions should be determined by each laboratory for each application</i>
Specificity	Human Cholesin
Calibration	Human Cholesin/C7ORF50 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 10%
Storage	2 - 8° C for 6 months, see page 2 for more information
This kit contains sufficient materials to run approximately 35-40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

Cholesin is a hormone produced in the intestine in response to dietary cholesterol absorption. It travels through the bloodstream to the liver, where it binds to a receptor called GPR146, which suppresses the liver’s cholesterol synthesis and helps lower circulating cholesterol levels.

High Sensitivity Human Cholesin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Cholesin from Human serum and plasma samples in a sandwich ELISA format. Mouse and or rat serum , plasma samples can NOT be detected by this ELISA.

The antibodies used in this ELISA Kit was validated specificity to human Cholesin by western blot, immunostaing. Results from this immunoassay have shown to accurately quantify Cholesin in human serum and plasma samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with an antibody specific for Cholesin. The capture antibody can bind to the Cholesin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against Cholesin is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of Cholesin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix reagents with those from other lots or sources.

_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Cholesin Microplate - 96 well microplate (12 strips of 8 wells) coated with an antibody against Cholesin.	027-06-01	1 plate
Cholesin Standard – 20 ng/vial of rh Cholesin in a buffered protein base with preservative; lyophilized.	027-06-02	1 vial
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrated of biotinylated antibody against Cholesin with preservative; lyophilized.	027-26-03	1 vial
Positive Control - one vial of recombinant Cholesin; lyophilized.	027-06-04	1 vial
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	DB01	1 bottle
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB92B	1 bottle
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB08C	1 bottle
Wash Buffer 20X - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution.	TMB01	1 bottle
Stop Solution - 11 mL of 0.125M HCl solution.	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 6 months. For longer storage up to 12 months, unopened

Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2-8° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Bioscience’s Order Code: 00700-01-25, 25 TIU for 50 ml sample solution) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application with a sample pretest.

Use polypropylene test tubes.

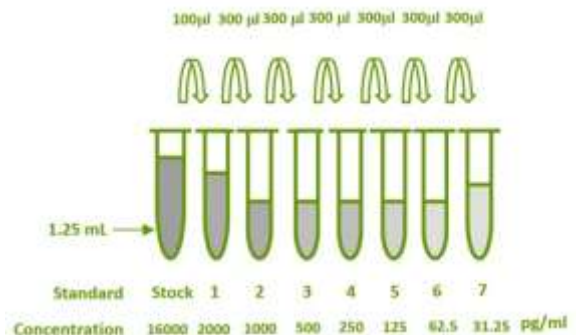
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute **25 mL of Wash Buffer Concentrate 20X** into deionized or distilled water (**475 mL**) to prepare 500 mL of 1x Wash Buffer.

Cholesin Standard - Reconstitute the Cholesin standard with 1.25 mL of Dilution Buffer. This reconstitution produces a stock solution of 16000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 300 µL of Dilution Buffer into the tube #2 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). Store the stock solution of standard at -20 ~ - 70 °C for a few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.25 ml	16000 pg/ml
# 1	100 µl of stock	700 µl	2000 pg/ml
# 2	300 µl of 1	300 µl	1000 pg/ml
# 3	300 µl of 2	300 µl	500 pg/ml
# 4	300 µl of 3	300 µl	250 pg/ml
# 5	300 µl of 4	300 µl	125 pg/ml
# 6	300 µl of 5	300 µl	62.5 pg/ml
# 7	300 µl of 6	300 µl	62.5 pg/ml



Positive Control - Reconstitute the Positive Control with 0.5 mL of Dilution Buffer. Discard the positive control after use. It is for one time use only.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody Diluent Solution (DB92B)** to produce a 10-fold concentrated stock solution. For 96 wells test, freshly pipette 9.45 mL of **Antibody Diluent Solution (DB92B)** into a 15 ml centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. *If run a partial strip test, freshly prepare 900 µL per strip (8-wells) of working solution. Store the stock solution of 10-fold concentrated detection antibody at -20 °C for a few days.*

Streptavidin-HRP Conjugate – For 96 wells test freshly pipette 11.88 mL of **HRP Diluent solution (DB08C)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution (**protect from light**).

The working solution of Streptavidin-HRP Conjugate should be freshly prepared and used within 10 min. If run a partial strip test, freshly prepare 900 µL per strip (8-wells) of working solution. Store the stock solution of 100-fold concentrated Streptavidin HRP ONLY at 2 -8°C for 12 months.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections. Pre-wash the Cholesin Microplate (027-09-01) by filling each well with 1x Wash Buffer (275 µL) using a channel-pipette. Discard the solution by hold plate and quickly invert plate top down.
2. Add 100 µL per well of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Discard the solution of each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a channel-pipette, or autowasher. Complete removal of liquid at each

step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 90 minutes on microplate shaker at room temperature.
6. Repeat the discard/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the discard/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 7 ~15 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 3 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve or 4-parameter fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human CHOLESIN /C7ORF50 recombinant	100
Mouse Cholesin recombinant	0
Human C5ORF24	0
Human sLOX-1	0

TYPICAL STANDARD CURVE

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.149)
31.25	0.046
62.5	0.104
125	0.207
250	0.409
500	0.793
1000	1.495
2000	2.278

Well Position



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Pre-wash Cholesin Microplate for 3 times prior to assay. Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate for 2 hours on the plate shaker at RT.
↓
Discard and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate for 90 minutes on the plate shaker at RT.
↓
Discard and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate for 45 min on the plate shaker at RT. Protect from light.
↓
Discard and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate 7-15 min on the plate shaker at RT. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read at 450 nm within 3 min.

Cholesin Proteins, Anti Human Cholesin Monoclonal Antibody and human Cholesin ELISA were manufactured by Aviscera Bioscience. We offer customer Services on Anti Mouse Cholesin Antibody and Mouse Cholesin ELISA Kit New Metabolism Research ELISA Kit were developed by Aviscera Bioscience.

- Human Feimin/C5ORF24 ELISA Kit SK00001-06
- Human Endotrophin ELISA Kit SK00009-08
- Mouse Raptin/RCN2 ELISA Kit SK00168-12
- Human Raptin/RCN2 ELISA Kit Sk00168-16
- Mouse BDNF ELISA Kit High Sensitivity SK00752-36
- Human LEAP-2 Mature ELISA Kit SK00007-06