ab108808 – Human Apolipoprotein CI ELISA Kit (Apo C1)

Instructions for Use

For the quantitative measurement of Human Apolipoprotein CI (Apo C1) in plasma and serum samples.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's Apolipoprotein CI (Apo C1) Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of apolipoprotein CI concentrations in plasma and serum samples.

An Apolipoprotein CI specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently an Apolipoprotein CI specific biotinylated detection antibody is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Complex is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of Apolipoprotein CI captured in plate.

Apolipoprotein CI is a 6.6 kDa apolipoprotein that is expressed primarily in the liver and activated when monocytes differentiate into macrophases. After being synthesized as a precursor with a length of 83 amino acids, apolipoprotein CI is processed to a single chain mature protein of 57 amino acids. It circulates in plasma and is a component of VLDL, IDL, and HDL. Apolipoprotein CI plays important modulatory roles in lipoprotein metabolism. It is an inhibitor of lipoprotein binding to the LDL receptor, LDL receptor-related protein, and VLDL receptor. It is the major plasma inhibitor of cholesteryl ester transfer protein and appears to interfere directly with fatty acid uptake. Apolipoprotein CI causes hypertriglyceridemia by inhibition of the lipoprotein lipase-dependent triglyceride-hydrolysis pathway. On the other hand, apolipoprotein CI is an activator of lecithin cholesterol acyl transferase that esterifies cholesterol and produces the formation of the mature HDL. It is also a physiological protector against infection by enhancing the early inflammatory response to lipopolysaccharide.

2. ASSAY SUMMARY

Primary capture antibody

Prepare all reagents, samples and standards as instructed.

Sample

Add standard or sample to each well used. Incubate at room temperature.

Primary detector antibody

Wash and add prepared biotin antibody to each well. Incubate at room temperature.

Streptavidin Label

Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.

Substrate   Colored product

Add Chromogen Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 4°C immediately upon receipt, apart from the Standard, SP Conjugate & Biotinylated Antibody, which should be stored at -20°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Apolipoprotein CI Microplate (12 x 8 well strips)	96 wells	4°C
Apolipoprotein CI Standard	2 x 1 vial	-20°C
10X Diluent M Concentrate	30 mL	4°C
Biotinylated Human Apolipoprotein CI Antibody	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	7 mL	4°C
Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Sealing Tapes	3	N/A

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 8 tubes to prepare standard or sample dilutions.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare fresh reagents immediately prior to use. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

9.1 **1X Diluent M**

Dilute the 10X Diluent M Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. *Store for up to 1 month at 4°C.*

9.2 **1X Wash Buffer**

Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

9.3 **1X Biotinylated Apolipoprotein CI Detector Antibody**

9.3.1 The stock Biotinylated Apolipoprotein CI Antibody must be diluted with 1X Diluent M according to the label concentration to prepare 1X Biotinylated Apolipoprotein CI Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated Apolipoprotein CI Antibody.

9.3.2 Calculate the necessary amount of 1X Diluent M to dilute the Biotinylated Apolipoprotein CI Antibody to prepare a 1X Biotinylated Apolipoprotein CI Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

Number of Wells Strips	Number of Wells	(V_T) Total Volume of 1X Biotinylated Antibody (µL)
4	32	1,760
6	48	2,640
8	64	3,520
10	80	4,400
12	96	5,280

Any remaining solution should be frozen at -20°C.

Where:

C_S = Starting concentration (X) of stock Biotinylated Apolipoprotein CI Antibody (variable)

C_F = Final concentration (always = 1X) of 1X Biotinylated Apolipoprotein CI Antibody solution for the assay procedure

V_T = Total required volume of 1X Biotinylated Apolipoprotein CI Antibody solution for the assay procedure

V_A = Total volume of (X) stock Biotinylated Apolipoprotein CI Antibody

V_D = Total volume of 1X Diluent M required to dilute (X) stock Biotinylated Apolipoprotein CI Antibody to prepare 1X Biotinylated Apolipoprotein CI solution for assay procedures

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells:

$$(C_F / C_S) \times V_T = V_A$$

Calculate the final volume of 1X Diluent M required to prepare the 1X Biotinylated Apolipoprotein CI Antibody:

$$V_T - V_A = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your antibody vial for the actual concentration of antibody provided.

C_S = 50X Biotinylated Apolipoprotein CI Antibody stock

C_F = 1X Biotinylated Apolipoprotein CI Antibody solution for use in the assay procedure

V_T = 3,520 µL (8 well strips or 64 wells)

$$(1X/50X) \times 3,520 \text{ µL} = 70.4 \text{ µL}$$

$$3,520 \text{ µL} - 70.4 \text{ µL} = 3,449.6 \text{ µL}$$

V_A = 70.4 µL total volume of (X) stock Biotinylated Apolipoprotein CI Antibody required

V_D = 3,449.6 µL total volume of 1X Diluent M required to dilute the 50X stock Biotinylated Antibody to prepare 1X Biotinylated Apolipoprotein CI Antibody solution for assay procedures

   9.3.3   First spin the Biotinylated Apolipoprotein CI Antibody vial to collect the contents at the bottom.

   9.3.4   Add calculated amount V_A of stock Biotinylated Apolipoprotein CI Antibody to the calculated amount V_D of 1X Diluent M. Mix gently and thoroughly.

9.4   **1X SP Conjugate**

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent M.

Any remaining solution should be frozen at -20°C.

10. STANDARD PREPARATIONS

- **Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.**

- **Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.**

- **This procedure prepares sufficient standard dilutions for duplicate wells.**

  10.1 Reconstitution of the Apolipoprotein CI Standard vial to prepare a 4 µg/mL Apolipoprotein CI **Standard #1**:

  10.1.1 First consult the Apolipoprotein CI Standard vial to determine the mass of protein in the vial.

  10.1.2 Calculate the appropriate volume of 1X Diluent M to add when resuspending the Apolipoprotein CI Standard vial to produce a 4 µg/mL Apolipoprotein CI **Standard #1** by using the following equation:

C_S = Starting mass of Apolipoprotein CI Standard (see vial label) (µg)

C_F = 4 µg/mL Apolipoprotein CI **Standard #1** final required concentration

V_D = Required volume of 1X Diluent M for reconstitution (µL)

Calculate total required volume 1X Diluent M for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 8 µg of Apolipoprotein CI Standard in vial

C_F = 4 µg/mL Apolipoprotein CI **Standard #1** final concentration

V_D = Required volume of 1X Diluent M for reconstitution

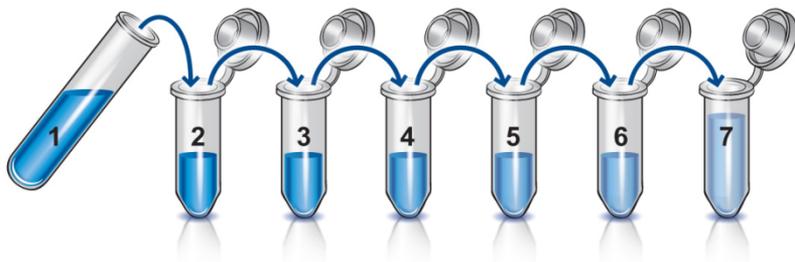$$(8\ \mu g\ /\ 4\ \mu g/mL)\ x\ 1{,}000 = 2{,}000\ \mu L$$

10.1.3 First briefly spin the Apolipoprotein CI Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the Apolipoprotein CI Standard vial by adding the appropriate calculated amount V_D of 1X Diluent M to the vial to generate the 4 µg/mL Apolipoprotein CI **Standard #1.** Mix gently and thoroughly.

10.2 Allow the reconstituted 4 µg/mL Apolipoprotein CI **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

10.3 Label seven tubes #2 – 8.

10.4 Add 120 µL of 1X Diluent M to tube #2 – 8.

10.5 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

10.6 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

10.7 Using the table below as a guide, prepare subsequent serial dilutions.

10.8 1X Diluent M serves as the zero standard 0 µg/mL (tube #8).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Volume Diluent M (µL)	Total Volume (µL)	Starting Conc. (µg/mL)	Final Conc. (µg/mL)
1		Step 10.1			4.000
2	120	120	240	4.000	2.000
3	120	120	240	2.000	1.000
4	120	120	240	1.000	0.500
5	120	120	240	0.500	0.250
6	120	120	240	0.250	0.125
7	120	120	240	0.125	0.063
8	-	120	120	-	0

11. SAMPLE PREPARATION

11.1 Plasma

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x *g* for 10 minutes. Dilute samples 1:100 into 1X Diluent M or within the range of 1:40 to 1:400. The undiluted samples can be stored at -20°C or below for up to 3 months Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as an anticoagulant).

11.2 Serum

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x *g* for 10 minutes. Dilute samples 1:100 into 1X Diluent M or within the range of 1:40 to 1:400. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

- Unused well plate strips should be returned to the plate packet and stored at 4°C.

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**

- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1   Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).

13.2   Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.

13.3   Add 50 μL of Apolipoprotein CI Standard or sample per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.

13.4   Wash five times with 200 μL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 μL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.

13.5   Add 50 μL of 1X Biotinylated Apolipoprotein CI Antibody to each well Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for two hours.

13.6   Wash microplate as described above.

13.7   Add 50 μL of 1X SP Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

13.8   Wash microplate as described above.

13.9   Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 30 minutes or until the optimal blue colour density develops.

13.10   Add 50 µL of Stop Solution to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. The color will change from blue to yellow.

13.11   Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

14. CALCULATIONS

Calculate the mean value of the triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Human Apo C1 Standard Curve

[H. Apo C1] (μg/ml)

$R^2 = 0.998$
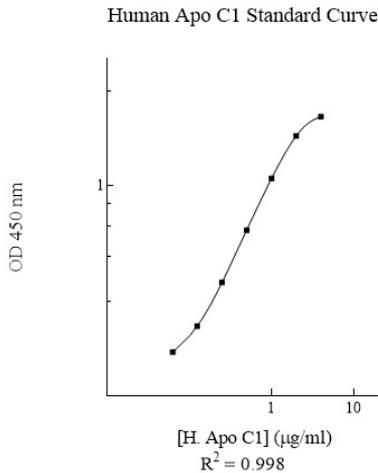
16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of Apolipoprotein CI is typically 36 ng/mL.

RECOVERY –

Standard Added Value: 0.25 – 2 μg/mL

Recovery %: 93 – 114.

Average Recovery %: 98

REFERENCE VALUES

Normal Human Apolipoprotein CI plasma levels range from 30 to 70 μg/ml.

LINEARITY OF DILUTION –

Plasma Dilution	Average % Expected Value
1:50	95
1:100	100
1:200	103

Serum Dilution	Average % Expected Value
1:50	94
1:100	101
1:200	105

PRECISION –

	Intra-Assay	Inter-Assay
% CV	5.5	10.9

17. ASSAY SPECIFICITY

Species	% Cross Reactivity
Canine	<2%
Bovine	None
Equine	None
Monkey	None
Mouse	None
Rat	None
Rabbit	None
Swine	None
Proteins	**% Cross Reactivity**
Human Apolipoprotein B	1%

No significant cross-reactivity observed with human Apo A1, Apo A2, Apo C2, Apo C3, and Apo E, Apo H and Apo M proteins.

18. <u>TROUBLESHOOTING</u>

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4°C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background/ Low sensitivity	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

19. NOTES

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)