



Human ApoC-II ELISA Kit

Vertrieb:

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Hinweis/Note:

Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Symbol Key

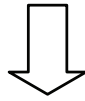


Consult instructions for use.

Assay Summary

Add 50 μ l of Standard/ Sample per well.

Incubate 2 hours.



Wash, then add
50 μ l of Biotinylated Antibody per well.

Incubate 1 hour.



Wash, then add
50 μ l of SP Conjugate per well.

Incubate 30 minutes.



Wash, then add
50 μ l of Chromogen Substrate per well.

Incubate 12 minutes.



Add 50 μ l of Stop Solution per well.

Read at 450 nm immediately.

Assay Template

	1								
A	2								
B	3								
C	4								
D	5								
E	6								
F	7								
G	8								
H	9								
	10								
	11								
	12								

AssayMax Human Apolipoprotein C-II ELISA Kit

Catalog No. EA8012-1
Sample Insert/Reference Only

Introduction

Apolipoprotein C-II (ApoC-II) is secreted in plasma and resides in both the very low density lipoproteins (VLDL) and high density lipoproteins (HDL). ApoC-II plays a major role in lipid metabolism as the obligate cofactor for lipoprotein lipase, which catalyzes the hydrolysis of triglyceride-rich lipoproteins (1). The protein has 79 amino acid residues and, in the absence of lipid, self-associates to form amyloid fibrils implicated in the pathogenesis of a number of diseases including Alzheimer's, Parkinson's, and Creutzfeldt-Jakob diseases (2). ApoC-II aggregates are present in human atherosclerotic plaques, and its fibrils initiate macrophage inflammatory responses (3). Deficiency of either apoC-II or lipoprotein lipase results in hypertriglyceridemia (4). Transgenic mice over-expression of human apoC-II also causes hypertriglyceridemia attributed to the delayed clearance of VLDL triglycerides (5).

Principle of the Assay

The AssayMax Human Apolipoprotein C-II ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human apoC-II in plasma, serum, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human apoC-II in less than 4 hours. A polyclonal antibody specific for human apoC-II has been pre-coated onto a 96-well microplate with removable strips. ApoC-II in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for apoC-II, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**

- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Human ApoC-II Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human apoC-II.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human ApoC-II Standard:** Human apoC-II in a buffered protein base (4 µg, lyophilized).
- **Biotinylated Human ApoC-II Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against Apo C-II (140 µl).
- **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 μ l, 20-200 μ l, 200-1000 μ l, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:200 into EIA Diluent or within the range of 1:50 - 1:800, and assay. The user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C and below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Dilute samples 1:200 into EIA Diluent or within the range 1:50 - 1:800, and assay. The user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C and below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 4 μ g of Human ApoC-II Standard with 1 ml of EIA Diluent to generate a 4 μ g/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (4 μ g/ml) 1:2 with equal volume of EIA Diluent to produce 2, 1, 0.5, 0.25, 0.125, and 0.063 μ g/ml solutions. EIA Diluent serves as the zero standard (0 μ g/ml). Any remaining solution should be frozen at -20°C and used within the next 30 days.

Standard Point	Dilution	[ApoC-II] ($\mu\text{g/ml}$)
P1	Standard (4 $\mu\text{g/ml}$)	4.000
P2	1 part P1 + 1 part EIA Diluent	2.000
P3	1 part P2 + 1 part EIA Diluent	1.000
P4	1 part P3 + 1 part EIA Diluent	0.500
P5	1 part P4 + 1 part EIA Diluent	0.250
P6	1 part P5 + 1 part EIA Diluent	0.125
P7	1 part P6 + 1 part EIA Diluent	0.063
P8	EIA Diluent	0.000

- **Biotinylated Human ApoC-II Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C .
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C .

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature ($20-25^{\circ}\text{C}$).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 μl of Human ApoC-II Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 μl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove liquid. If using a machine, wash six times with 300 μl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 μl of Biotinylated Human ApoC-II Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 μl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

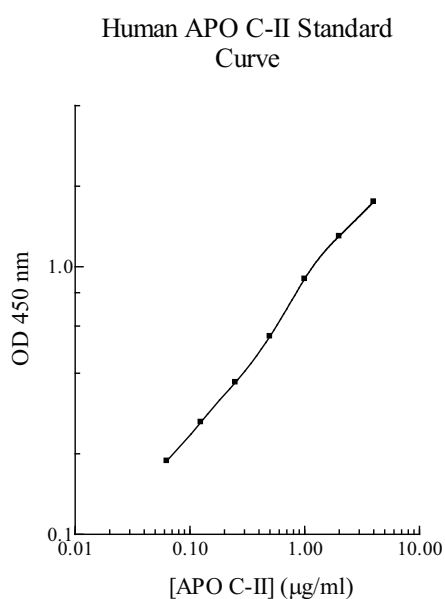
- Wash the microplate as described above.
- Add 50 μ l of Chromogen Substrate per well and incubate for 12 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing, and break the bubbles in the well with pipette tip.
- Add 50 μ l of Stop Solution to each well. The color will change from blue to yellow.
- 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.

Data Analysis

- Calculate the mean value of the duplicate or 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.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of ApoC-II is typically ~ 0.06 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0% and 7.5% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:100	89%	88%
1:200	98%	97%
1:400	104%	105%

Recovery

Standard Added Value	0.1 – 2.0 ug/ml
Recovery %	85 – 111%
Average Recovery %	96%

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	< 10%
Mouse	None
Rat	None
Swine	None

- No significant cross reactivity with ApoA-I, ApoA-II, ApoB, ApoC-I, ApoC-III or ApoE.

Reference Values

- Normal human ApoC-II plasma levels range from 20 – 100 µg/ml.

References

- (1) Jackson CL *et al.* (1984) *Proc. Natl. Acad. Sci. USA* 81:2945-2949
- (2) Hatters, DM *et al.* (2000) *Biochemistry* 39:8276-8283(5)
- (3) Medeiros LA *et al.* (2004) *J. Biol. Chem.* 279:10643-10648
- (4) Fojo SS and Brewer HB (1992) *J. Intern. Med.* 231:669-677
- (5) Shachter NS *et al.* (1994) *J. Clin. Invest.* 93:1683-1690

Version 3.6