Human Apolipoprotein C-II ELISA Kit (Plasma & Serum)

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).

Principal of the Assay

The Human ApoC-II ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human ApoC-II in plasma and serum samples. This assay employs a quantitative competitive enzyme immunoassay technique that measures human ApoC-II in less than 5 hours. A mAb capture antibody has been coated onto a 96-well microplate with removable strips. Monoclonal antibody against human ApoC-II binds to the captured antibody. ApoC-II in standards and samples is competed by a biotinylated ApoC-II sandwiched by the immobilized antibody and 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

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution

Reagents

- **mAb Capture Microplate:** A 96-well polystyrene mAb capture microplate (12 strips of 8 wells)
- **Monoclonal Antibody against Human Apo C-II (100x):** 1 vial (80µl)

- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Apo C-II Standard:** Human Apo C-II in a buffered protein base (40 µg, lyophilized).
- **Biotinylated Apo C-II (2x):** lyophilized, 1 vial
- **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).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 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

- Store kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Opened EIA Diluent may be stored for up to 1 month at 2-8^{0}C. Store reconstituted reagents at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000µl 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 2000 x g for 10 minutes and assay. Dilute samples 1:80 into EIA Diluent. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Dilute samples 1:80 into EIA Diluent. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Monoclonal Antibody against Human Apo C-II (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent. Any remaining solution should be frozen at -20^{0}C.
- **Standard Curve:** Reconstitute the 40 µg of Apo C-II Standard with 2 ml of EIA Diluent to generate a solution of 20 µg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the

standard solution (20 μg/ml) 1:2 with EIA Diluent to produce 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 μg/ml solutions. EIA Diluent serves as the zero standard (0 μg/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[Apo C-II] (μg/ml)
P1	1 part Standard (20 μg/ml) + 1 part EIA Diluent	10.000
P2	1 part P1 + 1 part EIA Diluent	5.000
P3	1 part P2 + 1 part EIA Diluent	2.500
P4	1 part P3 + 1 part EIA Diluent	1.250
P5	1 part P4 + 1 part EIA Diluent	0.625
P6	1 part P4 + 1 part EIA Diluent	0.313
P7	1 part P4 + 1 part EIA Diluent	0.156
P8	EIA Diluent	0.000

- **Biotinylated Apo C-II (2x):** Dilute Biotinylated Apo C-II with 4 ml EIA Diluent to produce a 2-fold solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to making dilution. The stock solution should be further diluted 1:2 with EIA Diluent. Any remaining solution should be frozen at -20^{0}C.
- **Wash Buffer Concentrate (20x):** 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^{0}C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- 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.
- Add 50μl of **Monoclonal Antibody against Human ApoC-II** to each well. Cover wells with a sealing tape and incubate for **two hours** at room temperature. Start the timer after the last sample addition.
- Wash five times with 200 μl of Wash Buffer. Invert the plate and decant the contents, and blot it on absorbent paper towel to completely remove liquid at each step.
- Add 25 μl of **Standard** and/or **Sample** per well, and immediately add 25 μl of **Biotinylated Apo C-II** to each well (on top of the standard or sample). Cover wells with a sealing tape and incubate for **two hours** at room temperature. Start the timer after the last sample addition.
- Wash five times with 200 μl of Wash Buffer. Invert the plate and decant the contents, and blot it on absorbent paper towel to completely remove liquid at each step.
- 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 five times with 200 μl of Wash Buffer.
- Add 50 μl of **Chromogen Substrate** per well and incubate for about **10 minutes** or until the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

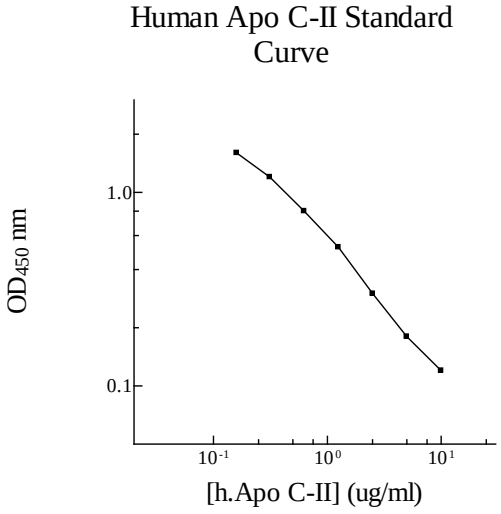
- Add 50 µl of **Stop Solution** to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately.

Data Analysis

- 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.

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 Apo C-II is typically 100 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8% and 7.4% respectively.
- No significant cross reactivity with Apo AI, Apo AII, Apo B, Apo CI, Apo CIII or Apo E.

Linearity

	Average Percentage of Expected Value
Sample Dilution	**Plasma/Serum**
1:40	97%
1:80	100%
1:160	105%

Recovery

Standard Added Value	0.5 – 2 ug/ml
Recovery %	84 - 116
Average Recovery %	100

Cross-Reactivity

Species	% Cross Reactivity
Beagle	< 1
Bovine	None
Monkey	< 10
Mouse	< 2
Rat	< 1
Swine	None

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

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