Human Apolipoprotein C-III ELISA Kit

Introduction

Apolipoprotein C-III (apoC-III) is a surface component of chylomicrons, very low density lipoproteins, and high density lipoproteins. It consists of 79 amino acids with a molecular mass of 8.8 kDa (1). ApoC-III is synthesized mainly in the liver and to a lesser degree in the intestine. It plays a key role in triglyceride-rich lipoprotein metabolism. It is an inhibitor of lipoprotein lipase and hepatic lipase, and interferes with binding of lipoproteins to cell surface heparan sulfate proteoglycans and receptors (2 -3). Overexpression of the human apoC-III gene causes hypertriglyceridemia in transgenic mice (4 - 5). Deficiency of apoC-III prevents hyperlipidemia induced by apoE overexpression (6). As its deficiency results in diet-induced obesity and aggravated insulin resistance in mice, apoC3 is a potential target for treatment of obesity and insulin resistance (7).

Principal of the Assay

The Human Apo C-III ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Apo C-III in plasma, serum, urine, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Apo C-III in less than 4 hours. A polyclonal antibody specific for human Apo C-III has been pre-coated onto a 96-well microplate with removable strips. Apo C-III in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Apo C-III, 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

- 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

- **Human Apo C-III Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Apo C-III.
- **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-III Standard:** Human Apo C-III in a buffered protein base (8 µg, lyophilized).
- **Biotinylated Apo C-III Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against Apo C-III (80 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 (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 1:500 into EIA Diluent as follows: add 10 µl of sample to 490 µl of EIA Diluent (1:50) to make Solution A; then add 80 µl of Solution A to 720 µl of EIA Diluent (1:10) to make a final working solution (1:500). Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA 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 1:500 into EIA Diluent as follows: add 10 µl of sample to 490 µl of EIA Diluent (1:50) to make Solution A; then add 80 µl of Solution A to 720 µl of EIA Diluent (1:10) to make a final working solution (1:500). Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. 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.
- **Standard Curve:** Reconstitute the 8 µg of Apo CIII standard with 4 ml of EIA Diluent to generate a stock solution of 2 µg/ml. Allow the standard to warm to room temperature prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (2 µg /ml) 1:4 with EIA Diluent to produce 0.5, 0.125, 0.0313 and 0.0078 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-III] (µg/ml)
P1	Standard (2 µg/ml)	2.0000
P2	1 part P1 + 3 part EIA Diluent	0.5000
P3	1 part P2 + 3 part EIA Diluent	0.1250
P4	1 part P3 + 3 part EIA Diluent	0.0313
P5	1 part P4 + 3 part EIA Diluent	0.0078
P6	EIA Diluent	0.0000

- **Biotin Apo C-III Antibody (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.
- **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 50 µl of Apo C-III standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
  If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated Apo C-III Antibody to each well and incubate for one hour.
- Wash a 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 a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 15 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 µl of Stop Solution to each well. The color will change from blue to yellow.

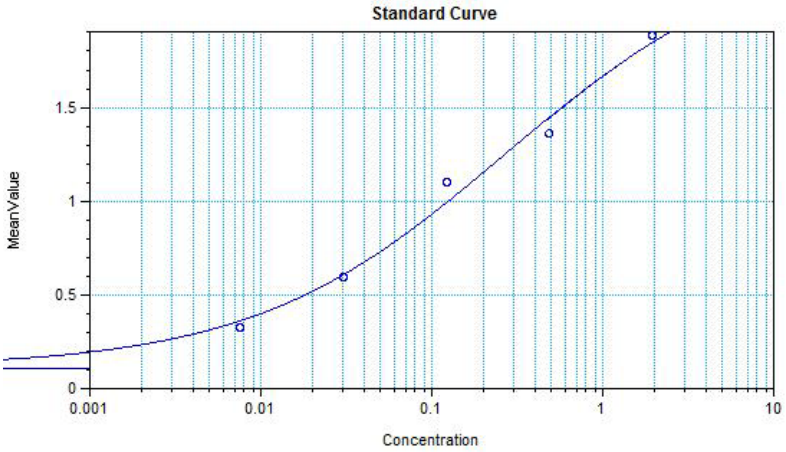
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be 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 Apo C-III is typically 5 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.3 % respectively.
- No significant cross reactivity with Apo AI, Apo AII, Apo B, Apo CI, Apo CII, or Apo E.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:250	103%	107%
1:500	102%	97%
1:1000	93%	96%

	Average Percentage of Expected Value
Sample Dilution	**Urine**
No Dilution	111%
1:2	94%
1:4	92%

Recovery

Standard Added Value	0.05 – 0.5 ug/ml
Recovery %	84 – 109 %
Average Recovery %	99 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	< 1
Bovine	None
Monkey	< 10 (suggest 1:10 dilution for plasma)
Mouse	< 2 (suggest 1:4 for plasma)
Rat	< 1
Swine	None

References

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(3) Dammerman M *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90: 4562–4566
(4) Ito Y *et al.* (1990) *Science* 249: 790-793
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