Human Apolipoprotein H ELISA Kit

Introduction

Apolipoprotein H (ApoH), previously known as β_2-glycoprotein I, is a 50 kDa plasma glycoprotein with 326 amino acids and circulates in plasma at about 200 μg/ml (1-4). ApoH inhibits the generation of factor Xa, XIa and XIIa, preventing activation of the intrinsic blood coagulation cascade (5-6). Binding of ApoH to anionic phospholipids such as phosphatidylserine and cardiolipin plays a key role in the formation of antiphospholipid antibodies, involving in autoimmune diseases like antiphospholipid syndrome or systemic lupus erythematosus (7-8). ApoH is increased in the plasma and liver of type 2 diabetic patients with metabolic syndrome and could be considered as a clinical marker of cardiovascular risk (9). ApoH interacts with viral proteins, such as the hepatitis B virus antigen, immunodeficiency virus type 1 and type 2 proteins, and Andes virus (10-11).

Principal of the Assay

The Human Apo H ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Apo H in plasma, serum, urine, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures Apo H in 4 hours. A polyclonal antibody specific for human Apo H has been pre-coated onto a 96-well microplate with removable strips. Apo H in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Apo H, 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 H Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Apo H.

- **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 H Standard:** Human Apo H in a buffered protein base (320 ng, lyophilized).
- **Biotinylated Apo H Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against Apo H (80 µl).
- **MIX 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

- Store kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Opened MIX 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 1:400 into MIX Diluent as follows: add 5 µl of sample to 495 µl of MIX Diluent (1:100) to make Solution A; then add 5 µl of Solution A to 995 µl of MIX Diluent (1:200) to make a final working solution (1:20 000). The undiluted samples can be stored 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 1:20 000 into MIX Diluent as follows: add 5 µl of sample to 495 µl of MIX Diluent (1:100) to make Solution A; then add 5 µl of Solution A to 995 µl of MIX Diluent (1:200) to make a final working solution (1:20 000). The undiluted samples can be stored 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 sample at 800 x g for 10 minutes. Dilute sample 1:16 into MIX Diluent and assay. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- **Saliva:** Collect saliva using sample tube. Centrifuge sample at 800 x g for 10 minutes. Dilute sample 1:16 into MIX Diluent 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.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Standard Curve:** Reconstitute the 320 ng of Apo H Standard with 4 ml of MIX Diluent to generate a solution of 80 ng/ml. 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 (80 ng/ml) 1:2 with MIX Diluent to produce 40, 20, 10, 5, 2.5, 1.25 and 0.625 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[Apo H] (ng/ml)
P1	1 part Standard (80 ng/ml) + 1 part MIX Diluent	40.00
P2	1 part P1 + 1 part MIX Diluent	20.00
P3	1 part P2 + 1 part MIX Diluent	10.00
P4	1 part P3 + 1 part MIX Diluent	5.000
P5	1 part P4 + 1 part MIX Diluent	2.500
P6	1 part P5 + 1 part MIX Diluent	1.250
P7	1 part P6 + 1 part MIX Diluent	0.625
P8	MIX Diluent	0.000

- **Biotin Apo H Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX 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 MIX 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 H 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.

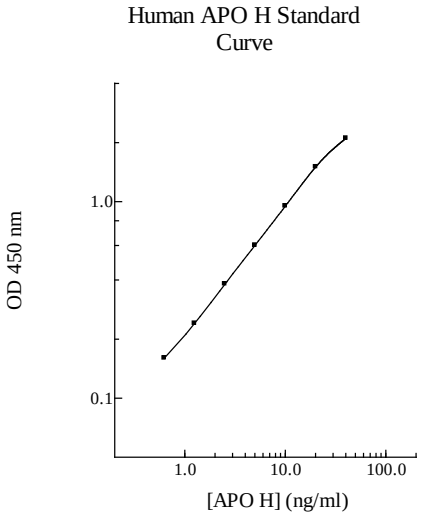
- Add 50 µl of Biotinylated Apo H Antibody to each well and incubate for one hour.
- Wash a microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 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 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**. Please note that after the reaction is stopped for about 10 minutes, some black particles may be generated at high concentration point, 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 H is typically 0.5 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.6 % and 7.7 % respectively.
- No significant cross reactivity with Apo AI, Apo AII, Apo B, Apo CI, Apo CII, or Apo CIII.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:10000	104%	102%
1:20000	100%	101%
1:40000	102%	100%

	Average Percentage of Expected Value	
Sample Dilution	**Urine**	**Saliva**
1:8	98%	101%
1:16	101%	100%
1:32	103%	104%

Recovery

Standard Added Value	0.05 – 0.5 ug/ml
Recovery %	88 - 110
Average Recovery %	99

Cross-Reactivity

Species	% Cross Reactivity
Beagle	< 0.1
Bovine	< 0.1
Monkey	< 5
Mouse	None
Rat	< 0.2
Rabbit	None
Swine	< 0.5

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