Human HDL ELISA Kit

Introduction

Human High-density lipoprotein (HDL) is the smallest and the densest of the discoidal and spherical lipoprotein particles. When fractionated by ultracentrifugation, HDL is separated into two major sub-fractions HDL2 (d 1.063–1.125 g/ml) and HDL3 (d 1.125–1.21 g/ml) (1-2). It contains 70% of apolipoprotein A-I, 20% of apolipoprotein A-II, phospholipids, and free cholesterol. HDL delivers cholesterol to liver cells which then secrete bile acids and cholesterol for excretion or re-utilization (3). HDL plays important anti-atherogenic roles, including cellular cholesterol efflux capacity, anti-oxidative, anti-inflammatory, antiapoptotic, vasodilatory, antithrombotic, and anti-infectious activities (4). Low plasma HDL cholesterol is an independent risk factor for the development of premature atherosclerosis. A rare form of genetic HDL deficiency is Tangier disease which is associated with mutations in the ATP-binding cassette transporter 1 gene (5).

Principal of the Assay

The HDL ELISA kit is designed for detection of HDL in plasma, serum, and cell culture supernatant. This assay employs a quantitative competitive enzyme immunoassay technique that measures HDL in less than 3 hours.
A polyclonal antibody specific for HDL has been pre-coated onto a 96-well microplate with removable strips. HDL in standards and samples is competed with a biotinylated HDL 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

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-protein, 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 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 acid solution.

Reagents

- **HDL Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against HDL.

- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **HDL Standard:** HDL in a buffered protein base (400 µg/ml, lyophilized).
- **Biotinylated HDL:** 1 vial, lyophilized.
- **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, 100x)**: 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 components of the kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Store SP Conjugate at -20^{0}C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8^{0}C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8^{0}C.
- Store Standard and Biotinylated Protein at 2-8^{0}C before reconstituting with Diluent and at -20^{0}C after reconstituting with Diluent.

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 human plasma 1:40 into EIA Diluent. 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. Remove serum and assay. Dilute human serum 1:40 into EIA Diluent. 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. The undiluted samples can be stored 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.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. 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 400 µg of HDL Standard with 1 ml of EIA Diluent to generate a solution of 400 µg/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 (400 µg/ml) 1:2 with equal volume of EIA Diluent to generate 200, 100, 50, 25, 12.5, and 6.25 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	[HDL] (µg/ml)
P1	1 part Standard Stock (400 µg/ml)	400.00
P2	1 part P1 + 1 part EIA Diluent	200.00
P3	1 part P2 + 1 part EIA Diluent	100.00
P4	1 part P3 + 1 part EIA Diluent	50.00
P5	1 part P4 + 1 part EIA Diluent	25.00
P6	1 part P5 + 1 part EIA Diluent	12.50
P7	1 part P6 + 1 part EIA Diluent	6.250
P8	EIA Diluent	0.000

- **Biotinylated HDL (1x):** Dilute Biotinylated HDL with 4 ml EIA Diluent to produce a working solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to use. Any remaining solution should be frozen at -20^{0}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^{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 25 µl of Standard and/or Sample per well, and immediately add 25 µl of Biotinylated HDL 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 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 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 µl of Chromogen Substrate per well and incubate for about 15 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. 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.

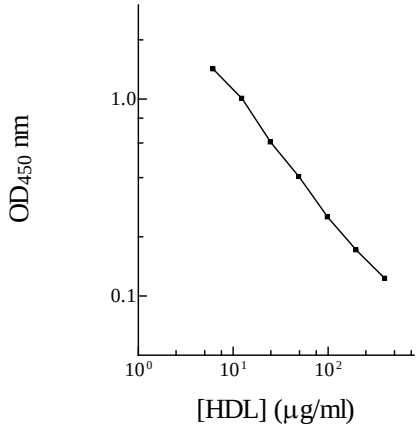
Data Analysis

- 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 four-parameter or log-log 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.

H. HDL Standard Curve



Performance Characteristics

- The minimum detectable dose of HDL is typically ~6 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9 % and 7.2 % respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:20	101%	99%
1:40	97%	91%
1:80	95%	92%

Recovery

Standard Added Value	10 - 100 µg/ml
Recovery %	85 - 104%
Average Recovery %	96 %

Reference Value

- The normal human plasma levels of HDL are 1-2 mg/ml.

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	15%
Mouse	5%
Rat	10%
Swine	1%
Rabbit	15%
Human	100%

Name	% Cross Reactivity
LDL	5%
IDL	10%
VLDL	10%

References

(1) Chapman MJ *et al.* (1981) *J. Lipid Res.* 22:339-358
(2) Barter P *et al.* (2003) *Atherosclerosis* 168(2):195-211
(3) Miller NE *et al*. (1985) *Nature* 314:109-111
(4) Kontush A and Chapman MJ (2006) Pharmacol Rev. 58(3):342-374
(5) Clee SM *et al.* (2001) *Circulation* 103(9):1198-1205

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