



Human ApoB ELISA Kit

Vertrieb:

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Hinweis/Note:

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

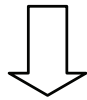
The datasheet is only a first information.
The relevant datasheet is included with the product.

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

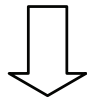
Thank you for choosing Assaypro.

Assay Summary

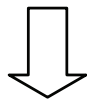
Add 50 μ l of Standard/ Sample per well.
Incubate 2 hours.



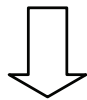
Wash, then add
50 μ l of Biotinylated Antibody per well.
Incubate 1 hour.



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 7 minutes.



Add 50 μ l of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

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

AssayMax Human Apolipoprotein B ELISA Kit

Catalog No. EA7001-1
Sample Insert/Reference Only

Introduction

Apolipoprotein B (ApoB) is the dominant protein constituent of LDL. The levels of secreted ApoB directly correlate with circulating serum cholesterol levels (1). ApoB is a better marker for risk of vascular disease than other lipid markers including LDL and HDL-cholesterol and triglycerides (2). ApoB is consistently associated with increased mortality in type 1 diabetes (3). VLDL and LDL with ApoB and plasma ApoB are independent risk factors for cardiovascular disease (CVD) (4). ApoB, ApoA-I, and the ApoA-I/ApoB ratio can predict incident ischemic stroke among patients with preexisting atherothrombotic disease (5).

Principle of the Assay

The AssayMax Human Apolipoprotein B ELISA (Enzyme-Linked Immunosorbent Assay) Kit is designed for detection of human ApoB in plasma, serum, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human ApoB in less than 4 hours. A polyclonal antibody specific for human ApoB has been pre-coated onto a 96-well microplate with removable strips. ApoB in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for ApoB, 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 ApoB Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human ApoB.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human ApoB Standard:** Human ApoB in a buffered protein base (2 µg, lyophilized).
- **Biotinylated Human ApoB Antibody (60x):** A 60-fold concentrated biotinylated polyclonal antibody against ApoB (120 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 µl, 20-200 µl, 200-1000 µl, 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 1:20000 into EIA Diluent or within the range of 1:10000 to 1:40000, and assay. The undiluted samples can be stored at -20°C or 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. Dilute samples 1:20000 into EIA Diluent or within the range of 1:10000 to 1:40000, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **CSF:** Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:5 into EIA Diluent and assay. The undiluted samples can be stored at -80°C 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.
- **Cell Lysate:** Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml cold PBS with 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Re-suspend pellet in ice-cold Lysis Buffer (10 mM Tris, pH8.0, 130 mM NaCl, 1% Triton X-100, protease inhibitor cocktail). For every 1×10^6 cells, add approximately 100 µL of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant for assay.

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 2 µg of Human ApoB Standard with 4 ml of EIA Diluent to generate a 0.5 µg/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 (0.5 µg/ml) 1:2 with EIA Diluent to produce 0.25, 0.125, 0.063, 0.031, 0.016, and 0.008 µg/ml solutions. EIA Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[ApoB] ($\mu\text{g/ml}$)
P1	1 part Standard (0.5 $\mu\text{g/ml}$) + 1 part EIA Diluent	0.250
P2	1 part P1 + 1 part EIA Diluent	0.125
P3	1 part P2 + 1 part EIA Diluent	0.063
P4	1 part P3 + 1 part EIA Diluent	0.031
P5	1 part P4 + 1 part EIA Diluent	0.016
P6	1 part P5 + 1 part EIA Diluent	0.008
P7	EIA Diluent	0.000

- **Biotinylated Human ApoB Antibody (60x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:60 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 ApoB 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 the 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 ApoB 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.

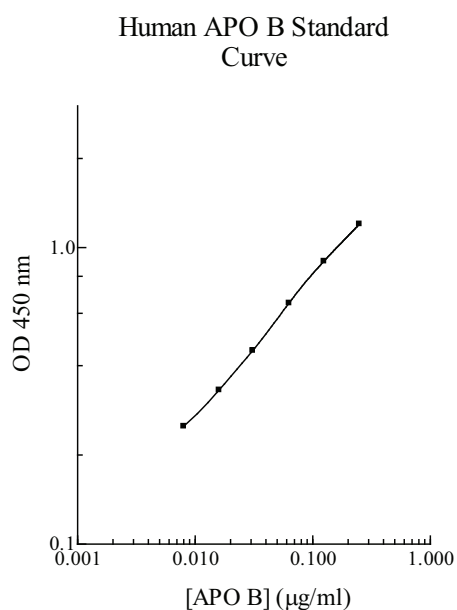
- Add 50 μ l of Chromogen Substrate per well and incubate for about 7 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μ l of Stop Solution to each well. The color will change from blue to 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 ApoB is ~ 0.0078 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.1% and 7.4% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:10000	90%	91%
1:20000	99%	98%
1:40000	102%	108%

Recovery

Standard Added Value	0.01 – 0.1 µg/ml
Recovery %	84 – 109%
Average Recovery %	98%

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	<5%
Mouse	None
Rat	None
Swine	None
Proteins	% Cross Reactivity
ApoA-I	<2%
ApoC-I	<10%

- No significant cross reactivity observed with ApoA-II, ApoC-II, ApoC-III, and ApoE.

Reference Values

- Normal human ApoB plasma levels range from 0.66 to 1.33 mg/ml.

References

- (1) Brodsky JL *et al.* (2008) *Trends Endocrinol Metab.* Sep;19(7):254-9.
- (2) Adiloglu AK *et al.* (2005) *Acta Cardiol.* Dec;60(6):599-604.
- (3) Stettler C *et al.* (2006) *J Intern Med.* Sep;260(3):272-80.
- (4) Furtado JD *et al.* (2008) *Am J Clin Nutr.* Jun;87(6):1623-30.
- (5) Koren-Morag N *et al.* (2008) *J Neurol Sci.* Jul 15;270(1-2):82-7.

Version 3.4