Quantikine® ELISA

Human Apolipoprotein B/ApoB Immunoassay

Catalog Number DAPB00

For the quantitative determination of human Apolipoprotein B (ApoB) concentrations in serum and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Apolipoprotein B (ApoB) is a 550 kDa palmitoylated and variably glycosylated member of the large lipid transfer (LLT) superfamily (1). It is a major protein component of chylomicron, very low density lipoprotein (VLDL), and low density lipoprotein (LDL) particles. Apolipoprotein-based particles are utilized to transport cholesterol and triglycerides in the circulation. The determination of circulating ApoB levels is an important component of risk assessment for cardiovascular disease, insulin resistance, and metabolic syndrome. ApoB levels are relatively stable compared to LDL and VLDL levels which rise following eating and require patient fasting before accurate baseline values can be determined.

Mature full length human ApoB (ApoB100) shares approximately 70% amino acid (aa) sequence identity with comparable regions of mouse and rat ApoB (2-6). ApoB48 corresponds to the N-terminal 48% (2119 aa) of the protein and is generated by insertion of a premature stop codon in the ApoB transcript (7, 8). In humans, ApoB100 is the predominant form expressed in the liver while ApoB48 predominates in the small intestine (7-9). Additional minor forms are produced by premature termination and are also named by the percentage of the full length protein they contain. One of these, ApoB37, is produced in familial hypobetalipoproteinemia which is characterized by low ApoB secretion and low circulating LDL-cholesterol levels (10). Fragments of ApoB can also be generated by proteolytic degradation (11).

Dietary lipids are absorbed by small intestine enterocytes which package them with ApoB into chylomicrons for release into the circulation. Endogenous lipids synthesized in the liver are packaged with ApoB into VLDL particles and released into the serum. ApoB is required for the assembly and secretion of VLDL particles and chylomicrons (12-15). In the endoplasmic reticulum, ApoB is loaded with lipids by microsomal triglyceride transfer protein (MTP). MTP is one of several phospholipid transfer proteins (PLTPs) and can remain associated with lipoprotein particles in the circulation (16). Liver PLTP activity is upregulated in hyperinsulinemia and obesity (12). Its plasma levels are negatively associated with plasma HDL and positively associated with plasma triglycerides, ApoB, and ApoE levels (14, 17).

Circulating ApoB-containing lipoprotein particles deliver lipids and cholesterol to tissues via interactions with lipoprotein lipase (LPL) on the surface of endothelial cells (18, 19). ApoB is retained in the depleted particles which are reduced in size and known as VLDL remnants or chylomicron remnants. ApoB-lipoprotein particle remnants are cleared by receptor mediated endocytosis in the liver by LDLR and by hepatic lipase on the cell surfaces of hepatocytes and sinusoid cells (18, 20, 21). LPL itself can be incorporated into ApoB lipoproteins where it enhances receptor-mediated particle uptake (22). ApoB-containing particles can also penetrate the vascular endothelium and initiate atherogenesis, plaque formation, and inflammation (23, 24).

The Quantikine Human Apolipoprotein B/ApoB Immunoassay is a 4.5 hour solid phase ELISA designed to measure ApoB levels in serum and plasma.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human ApoB has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ApoB present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human ApoB is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of ApoB bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Samples must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

<table>
<tr><th>PART</th><th>PART #</th><th>DESCRIPTION</th><th>STORAGE OF OPENED/
RECONSTITUTED MATERIAL</th></tr>
<tr><td>Human ApoB Microplate</td><td>894224</td><td>96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human ApoB.</td><td>Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Human ApoB Standard</td><td>894226</td><td>2 vials of natural human ApoB in a buffer with preservatives; lyophilized. Refer to the vial label for reconstitution volume.</td><td>Discard after use.
Use a fresh standard for each assay.</td></tr>
<tr><td>Human ApoB Conjugate</td><td>894225</td><td>21 mL of a polyclonal antibody specific for human ApoB conjugated to horseradish peroxidase with preservatives.</td><td rowspan="7">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Assay Diluent RD1-113</td><td>895983</td><td>21 mL of a buffered protein base with preservatives.</td></tr>
<tr><td>Calibrator Diluent RD5-61</td><td>895984</td><td>21 mL of a concentrated buffered protein base with blue dye and preservatives.
May contain a gel. Used diluted 1:5 in this assay.</td></tr>
<tr><td>Wash Buffer Concentrate</td><td>895003</td><td>21 mL of a 25-fold concentrated solution of buffered surfactant with preservative.
May turn yellow over time.</td></tr>
<tr><td>Color Reagent A</td><td>895000</td><td>12 mL of stabilized hydrogen peroxide.</td></tr>
<tr><td>Color Reagent B</td><td>895001</td><td>12 mL of stabilized chromogen (tetramethylbenzidine).</td></tr>
<tr><td>Stop Solution</td><td>895032</td><td>6 mL of 2 N sulfuric acid.</td></tr>
<tr><td>Plate Sealers</td><td>N/A</td><td colspan="2">4 adhesive strips.</td></tr>
</table>

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12″ orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The ApoB Standard provided in this kit was derived from human blood. The source material was tested at the donor level using FDA licensed methods and found to be non-reactive for anti-HIV-1/2, anti-HCV, and Hepatitis B surface antigen. As no testing can offer complete assurance of freedom from infectious agents, the Standard should be handled as if capable of transmitting infection.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 1000-fold dilution. A suggested 1000-fold dilution can be achieved by adding 25 μL of sample to 475 μL of Calibrator Diluent RD5-61 (diluted 1:5).* Complete the 1000-fold dilution by adding 20 μL of the diluted sample to 980 μL of Calibrator Diluent RD5-61 (diluted 1:5).

*See Reagent Preparation section.

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REAGENT PREPARATION

Bring all reagents to room temperature before use.

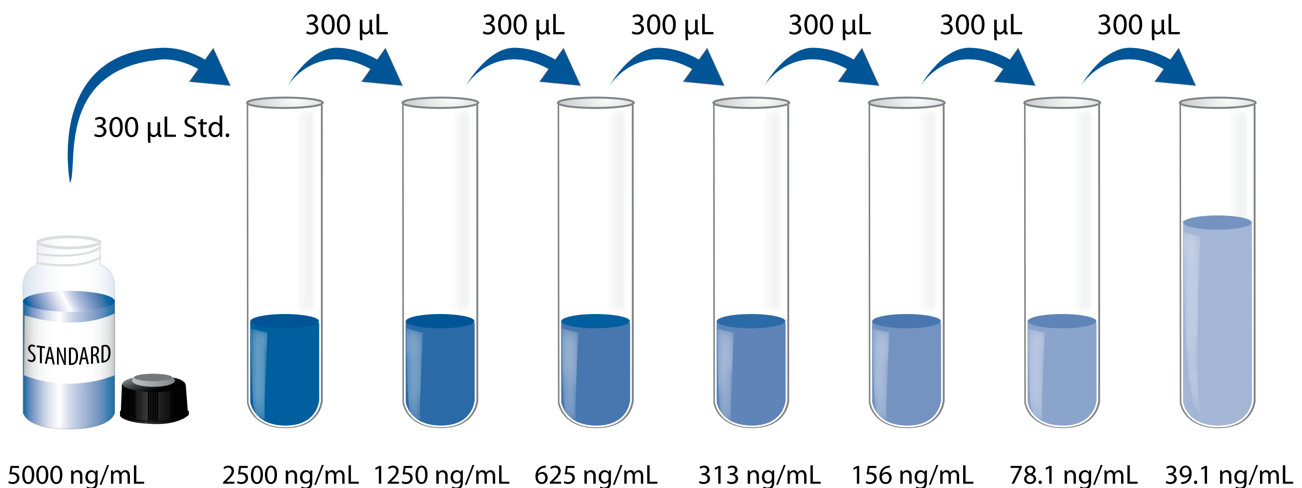
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μL of the resultant mixture is required per well.

Calibrator Diluent RD5-61 (diluted 1:5) - *If diluent contains a gel, heat to 37 °C and mix well until the gel has completely dissolved.* Add 20 mL of Calibrator Diluent RD5-61 to 80 mL of deionized or distilled water to yield 100 mL of Diluted Calibrator Diluent RD5-61 (diluted 1:5).

Human ApoB Standard - **Refer to vial label for reconstitution volume.** Reconstitute the Human ApoB Standard with deionized or distilled water. This reconstitution produces a stock solution of 5000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 300 μL of Calibrator Diluent RD5-61 (diluted 1:5) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2500 ng/mL standard serves as the high standard. Calibrator Diluent RD5-61 (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 200 µL of Assay Diluent RD1-113 to each well.
4. Add 50 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12″ orbit) set at 500 rpm ± 50 rpm.

   Note: *Pipette Standard and samples within 15 minutes.*
5. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper toweling.
6. Add 200 µL of Human ApoB Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

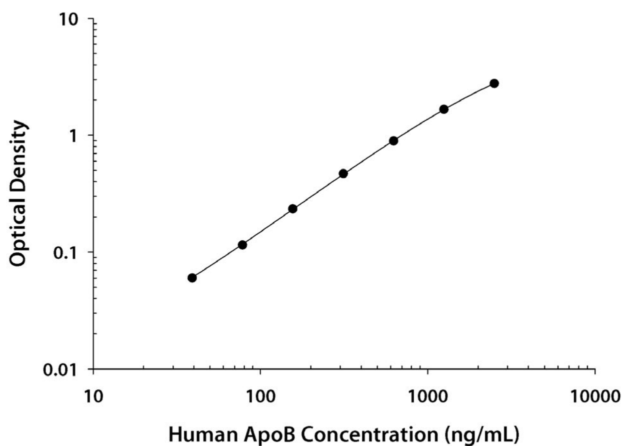
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human ApoB concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.017 0.018	0.018	—
39.1	0.077 0.079	0.078	0.060
78.1	0.131 0.134	0.133	0.115
156	0.245 0.258	0.252	0.234
313	0.480 0.493	0.487	0.469
625	0.901 0.920	0.911	0.893
1250	1.642 1.713	1.678	1.660
2500	2.746 2.833	2.790	2.772

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/mL)	284	654	1228	261	717	1375
Standard deviation	15.0	33.6	48.1	27.4	61.2	90.5
CV (%)	5.3	5.1	3.9	10.5	8.5	6.6

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human ApoB were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	100	103	105
	Range (%)	94-112	96-106	92-115
1:4	Average % of Expected	102	109	105
	Range (%)	86-114	102-118	93-113
1:8	Average % of Expected	95	100	103
	Range (%)	82-117	93-107	97-109
1:16	Average % of Expected	100	105	104
	Range (%)	86-115	92-113	101-110

*Samples were diluted prior to assay.

SENSITIVITY

Thirty-two assays were evaluated and the minimum detectable dose (MDD) of human ApoB ranged from 0.06-9.97 ng/mL. The mean MDD was 2.69 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against highly purified ApoB from human plasma.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human ApoB in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (μg/mL)	Range (μg/mL)	Standard Deviation (μg/mL)
Serum (n=33)	592	189-991	166
EDTA plasma (n=33)	568	189-985	164
Heparin plasma (n=33)	594	195-1033	174

SPECIFICITY

This assay recognizes natural human ApoB.

The factors listed below were prepared at 2500 ng/mL in Calibrator Diluent and assayed for cross reactivity. Preparations of the following factors at 2500 ng/mL in a mid-range human ApoB control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ApoA1
ApoA2
ApoC1
ApoC2
ApoC3
ApoD
ApoE
ApoE3
ApoER2
ApoH
ApoJ
ApoM

Recombinant mouse:

ApoH

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