PRODUCT INFORMATION & MANUAL

Human Apolipoprotein B/ApoB Valukine™ ELISA

Catalog Number: VAL214

For the quantitative determination of natural human
Apolipoprotein B (ApoB) concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202411.1

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I. BACKGROUND

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).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

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

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for human serum and human plasma.
- 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.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent (1×) and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
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

B. 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.

C. CALIBRATION

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

D. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human ApoB and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution		Human Serum (n=4)	Human EDTA plasma (n=4)	Human 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

E. SAMPLE VALUES

Human 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)
Human Serum (n=33)	592	189-991	166
Human EDTA plasma (n=33)	568	189-985	164
Human Heparin plasma (n=33)	594	195-1033	174

F. SPECIFICITY

This assay recognizes natural human ApoB.

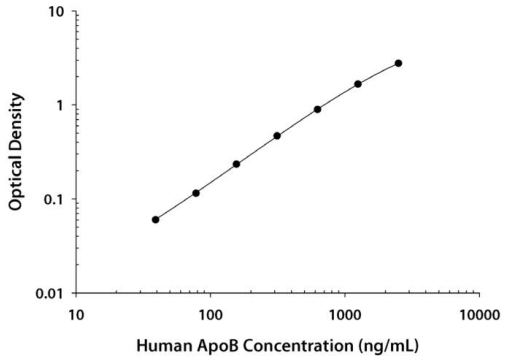
The factors listed below were prepared at 2500 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 2500 ng/mL in a mid-range human ApoB control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human：	**Recombinant mouse:**
ApoA1	ApoH
ApoA2	
ApoC1	
ApoC2	
ApoC3	
ApoD	
ApoE	
ApoE3	
ApoER2	
ApoH	
ApoJ	
ApoM	

IV. EXPERIMENT

EXAMPLE STANDARD

The 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

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human ApoB Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human ApoB	1 plate
Human ApoB Conjugate	Solution of antibody against human ApoB conjugated to horseradish peroxidase	1 vial
Human ApoB Standard	Natural human ApoB in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	2 vials
Assay Diluent RD1-113	A buffered protein base	1 vial
Calibrator Diluent Concentrate (5×)/ RD5-61	A 5× concentrated buffered protein base used to dilute standard and samples	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Sealers	Adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="7">Opened/
Reconstituted
Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>Use a fresh standard for each assay.
Discard after use.</td></tr>
<tr><td>Assay Diluent
RD1-113</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent
Concentrate (5×)/
RD5-61</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

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

C. 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 cylinder.
- Horizontal orbital shaker (0.12” orbit) capable of maintaining a speed of 500±50 rpm
- Test tubes for dilution of standards and samples.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
- 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.

VI. PREPARATION

A. SAMPLE COLLECTION AND 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 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

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

B. SAMPLE PREPARATION

Human serum and plasma samples recommend a 1000-fold dilution. A suggested 1000-fold dilution can be achieved by adding 25 µL of sample to 475 µL of Calibrator Diluent (1×). Complete the 1000-fold dilution by adding 20 µL of the diluted sample to 980 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

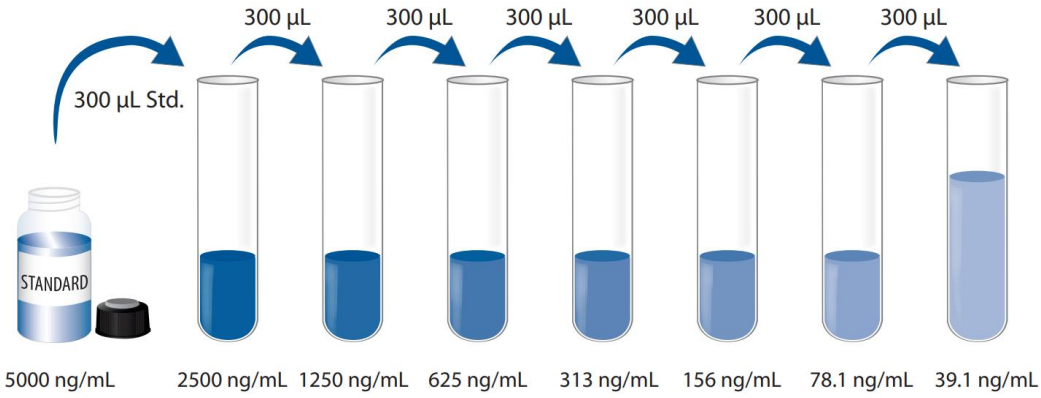
Calibrator Diluent (1×) - *If diluent contains a gel, heat to 37 °C and mix well until the gel has completely dissolved.* Use deionized or distilled water to prepare Calibrator Diluent (1×).

Human ApoB Standard - Refer to the vial label for the 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.

*If you have any question, please seek help from our Technical Support.

Pipette 300 µL of Calibrator Diluent (1×) 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 (1×) serves as the zero standard (0 ng/mL).



D. 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.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB Substrate. 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 TMB Substrate.

VII.ASSAY PROCEDURE

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

1. Prepare all reagents and working standards 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 and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital shaker (0.12" orbit) set at 500±50 rpm.** A plate layout is provided for a record of standards and samples assayed.

   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 towels.
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 a horizontal orbital shaker (0.12" orbit) set at 500±50 rpm.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 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.
11. **CALCULATION OF RESULTS**

    Average the duplicate readings for each standard 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.

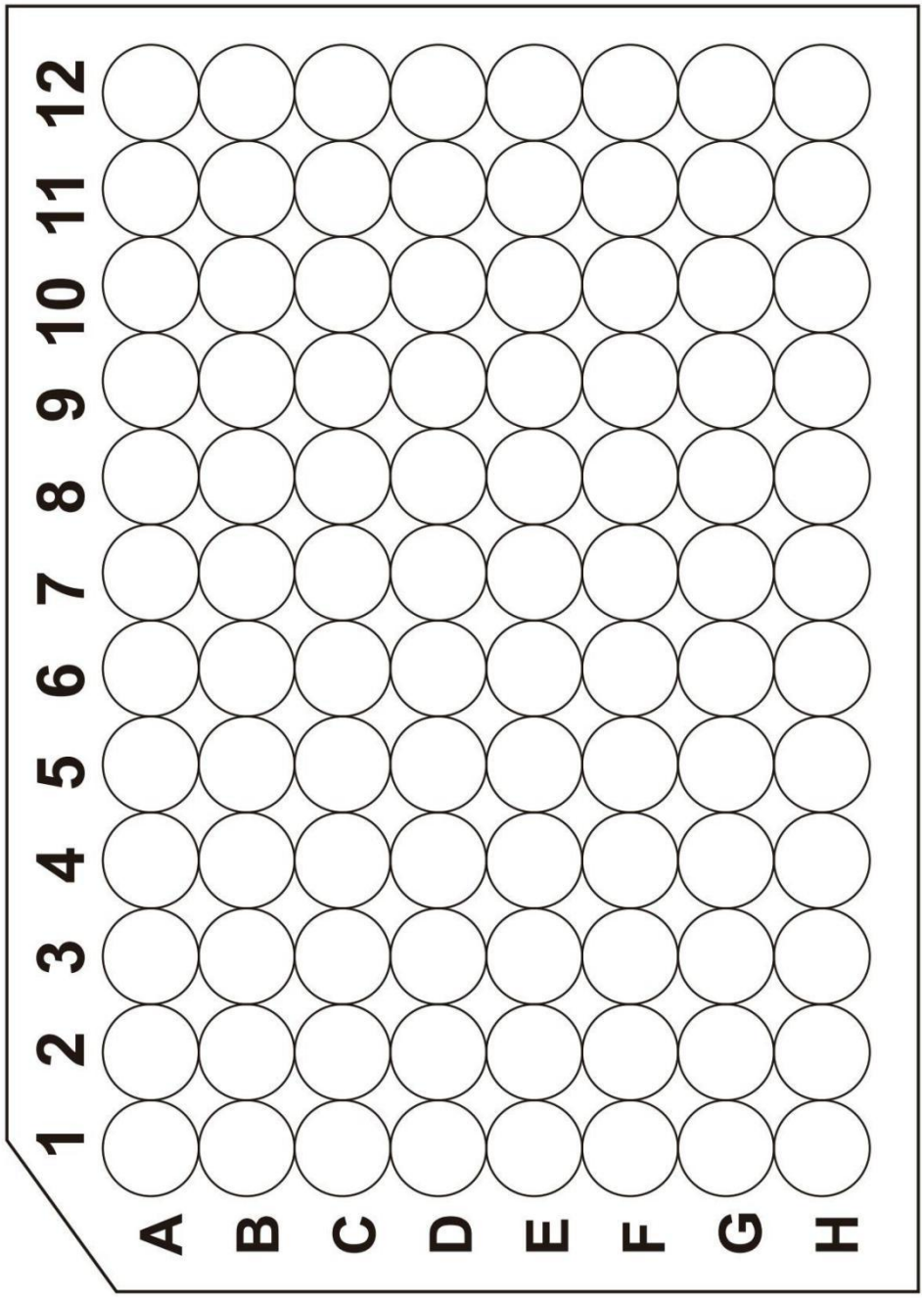
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Sniderman, A.D. and S.M. Marcovina (2006) Clin. Lab. Med. 26:733.
2. Law, S.W. et al. (1986) Proc. Natl. Acad. Sci. USA 83:8142.
3. Chen, S.H. et al. (1986) J. Biol. Chem. 261:12918.
4. Yang, C.Y. et al. (1986) Nature 323:738.
5. Knott, T.J. et al. (1986) Nature 323:734.
6. Cladaras, C. et al. (1986) EMBO J. 5:3495.
7. Chen, S.H. et al. (1987) Science 238:363.
8. Powell, L.M. et al. (1987) Cell 50:831.
9. Higuchi, K. et al. (1988) Proc. Natl. Acad. Sci. USA 85:1772.
10. Young, S.G. et al. (1988) Science 241:591.
11. Cardin, A.D. et al. (1984) J. Biol. Chem. 259:8522.
12. Jiang, X.C. et al. (2001) Nat. Med. 7:847.
13. Yazdanyar, A. and X.C. Jiang (2012) Hepatology 56:576.
14. Moerland, M. et al. (2008) Arterioscler. Thromb. Vasc. Biol. 28:1277.
15. Zhao, Y. et al. (2000) Mol. Biol. Cell 11:721.
16. Quintao, E.C.R. and P.M. Cazita (2010) Atherosclerosis 209:1.
17. Chen, X. et al. (2009) Nutr. Metab. 6:49.
18. Choi, S. et al. (1995) J. Biol. Chem. 270:8081.
19. Boren, J. et al. (2001) J. Biol. Chem. 276:26916.
20. Dichek, H.L. et al. (2004) J. Lipid Res. 45:551.
21. Dichek, H.L. et al. (1998) J. Biol. Chem. 273:1896.
22. Zheng, C.Y. et al. (2006) Arterioscler. Thromb. Vasc. Biol. 26:891.
23. Segrest, J.P. (2002) Curr. Diab. Rep. 2:282.
24. Proctor, S.D. et al. (2002) Curr. Opin. Lipidol. 13:461.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人载脂蛋白 B/ApoB Valukine™ ELISA 试剂盒

目录号：VAL214

适用于定量检测天然人载脂蛋白 B（ApoB）的浓度

科研专用，不可用于临床诊断

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版本号 202411.1

目录

I. 背景

载脂蛋白 B（Apolipoprotein B，ApoB）是一种 550 kDa 的棕榈酰化和可变糖基化的大脂转移（LLT）超家族成员（1）。它是乳糜微粒、极低密度脂蛋白（VLDL）和低密度脂蛋白（LDL）颗粒的主要蛋白质成分。载脂蛋白颗粒在血液循环被用于中运输胆固醇和甘油三酯。测定循环 ApoB 水平是心血管疾病、胰岛素抵抗和代谢综合征风险评估的重要组成部分。ApoB 的水平相对稳定，而 LDL and VLDL 的水平会在进食后升高，需要患者禁食后才能确定准确的基线值。

成熟的全长人 ApoB（ApoB100）与小鼠和大鼠 ApoB 的类似区域具有约 70%的氨基酸（aa）序列相同性（2-6）。ApoB48 对应于蛋白的 N 端 48%（2119 aa），是通过在 ApoB 转录本中插入过早终止密码子产生的（7, 8）。在人体中，ApoB100 主要在肝脏中表达，而 ApoB48 则主要在小肠中表达（7-9）。其他次要形式由提前终止产生，也按其所含全长蛋白质的百分比命名。其中一种 ApoB37 是在家族性低脂蛋白血症中产生的，这种血症的特点是 ApoB 分泌低和循环中 LDL-胆固醇水平低（10）。蛋白水解也会产生 ApoB 片段（11）。

食物中的脂质被小肠肠细胞吸收，肠细胞将脂质与 ApoB 一起包装成乳糜微粒，释放到血液循环中。在肝脏中合成的内源性脂质与 ApoB 一起被包装成 VLDL 颗粒，并释放到血清中。VLDL 颗粒和乳糜微粒的组装和分泌都需要 ApoB（12-15）。在内质网中，ApoB 通过微粒体甘油三酯转移蛋白（MTP）载入脂质。MTP 是几种磷脂转移蛋白（PLTPs）之一，可在循环中与脂蛋白颗粒保持关联（16）。肝脏 PLTP 活性在高胰岛素血症和肥胖症中上调（12）。其血浆水平与血浆 HDL 呈负相关，与血浆甘油三酯 ApoB 和 ApoE 水平呈正相关（14, 17）。

循环中含有 ApoB 的脂蛋白颗粒通过与内皮细胞表面的脂蛋白脂肪酶（LPL）相互作用，将脂质和胆固醇输送到组织中（18, 19）。ApoB 保留在被耗尽的微粒中，这些微粒体积变小，被称为 VLDL 残余物或乳糜微粒残余物。ApoB -脂蛋白颗粒残余在肝脏中通过 LDLR 受体介导的内吞作用以及肝细胞和窦状细胞表面的肝脂肪酶清除（18, 20, 21）。LPL 本身可融入 ApoB 脂蛋白中，从而增强受体介导的颗粒摄取（22）。含 ApoB 的颗粒还能穿透血管内皮，引发动脉粥样硬化、斑块形成和炎症（23, 24）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人ApoB抗体包被于微孔板上，样品和标准品中的人ApoB会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人ApoB检测抗体进行孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于人血清和人血浆；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液（1×）稀释并重复测定。
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 （ng/mL）	284	654	1228	261	717	1375
标准差	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

B. 灵敏度

评估了 32 次测定，人 ApoB 的最小可检测剂量（MDD）范围为 0.06-9.97 ng/mL。平均 MDD 为 2.69 ng/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

C. 校正

该免疫测定法以高纯度的来自人血浆的人ApoB校正。

D. 线性

不同的样本中含有或掺入高浓度的人ApoB，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数		人血清（n=4）	人EDTA血浆（n=4）	人肝素血浆（n=4）
1:2	平均值/期待值（%）	100	103	105
	范围（%）	94-112	96-106	92-115
1:4	平均值/期待值（%）	102	109	105
	范围（%）	86-114	102-118	93-113
1:8	平均值/期待值（%）	95	100	103
	范围（%）	82-117	93-107	97-109
1:16	平均值/期待值（%）	100	105	104
	范围（%）	86-115	92-113	101-110

E. 样本预值

人血清/血浆–在本试验中评估了来自表面健康志愿者的样本中是否存在人 ApoB。本研究中使用的供体没有病史。

样本类型	平均值（μg/mL）	范围（μg/mL）	标准偏差（μg/mL）
人血清（n=33）	592	189-991	166
人EDTA血浆（n=33）	568	189-985	164
人肝素血浆（n=33）	594	195-1033	174

F. 特异性

检测方法可检测天然人ApoB。

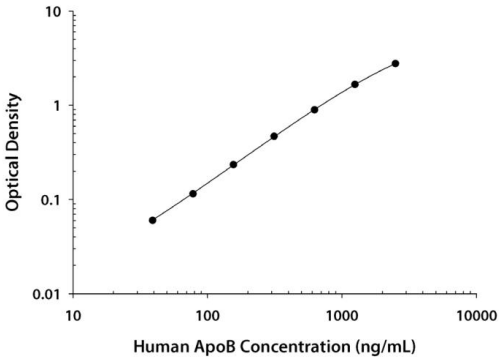
以下列出的蛋白在标准品稀释液（1×）中以2500 ng/mL的浓度制备，并进行交叉反应性测定。以下列出的蛋白在中值范围人ApoB对照品中以2500 ng/mL的浓度制备，并进行干扰测定。未观察到明显的交叉反应或干扰。

Recombinant human：	**Recombinant mouse:**
ApoA1	ApoH
ApoA2	
ApoC1	
ApoC2	
ApoC3	
ApoD	
ApoE	
ApoE3	
ApoER2	
ApoH	
ApoJ	
ApoM	

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



(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

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human ApoB Microplate	包被抗人ApoB抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human ApoB Conjugate	酶标检测抗人ApoB抗体	1瓶
Human ApoB Standard	天然人ApoB标准品（冻干），参考瓶身标签进行重溶	2瓶
Assay Diluent RD1-113	检测液	1瓶
Calibrator Diluent Concentrate (5×)/ RD5-61	浓缩的标准品稀释液（5×），用于稀释标准品和样品	1瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8°C储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8°C储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>每次使用新的标准品，用后丢弃。</td></tr>
<tr><td>检测液RD1-113</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>浓缩的标准品稀释液（5×）/ RD5-61</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的1×标准品稀释液，多余的丢弃</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；
2-8 °C储存，最多30天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm检测波长的吸收值及540 nm或570 nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 100 mL和500 mL量筒
- 水平振荡器（0.12”轨道），转速：500±50 rpm
- 用于稀释标准品和样品的管子。

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。
- 试剂盒中提供的ApoB标准品源自人血。使用FDA许可的方法在供体水平对原材料进行了检测，发现其对抗HIV-1/2、抗HCV和乙型肝炎表面抗原无反应。由于任何检测都不能完全保证无传染性病原体，应将标准品视为可能传播感染的物质。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

血清 - 使用血清分离管（SST），让样本在室温下凝固30分钟，然后在1000 × g的离心力下离心15分钟。分离血清并立即进行检测，或分装并储存在≤ -20 °C。避免反复冻融循环。样本可能需要用标准品稀释液（1×）稀释。

血浆 - 使用肝素或EDTA作为抗凝剂收集血浆。在采样后30分钟内，以1000 × g的离心力离心15分钟。立即检测或分装并储存在≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

注：*柠檬酸盐血浆在本检测中未经验证。*

B. 样品准备

人血清和血浆样本建议稀释 1000 倍。建议的 1000 倍稀释方法为：将 25 µL 的样本加入 475 µL 的标准品稀释液（1×）。取 20 µL 该稀释的样本加入到 980 µL 的标准品稀释液（1×）中完成 1000 倍稀释。最佳稀释倍数应由用户自行确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

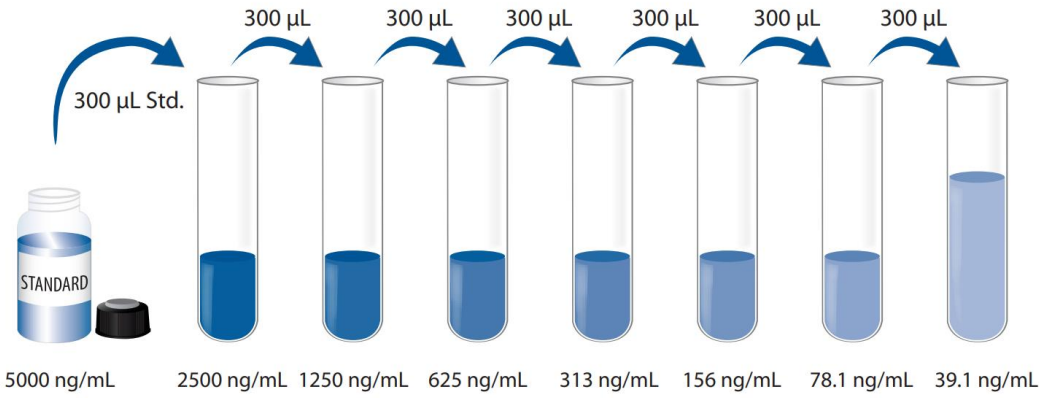
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）：*如果稀释液含有凝胶，加热至37°C，并充分混合，直至凝胶完全溶解。*使用去离子水或蒸馏水制备标准品稀释液（1×）。

人ApoB标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水重构人ApoB标准品，得到浓度为5000 ng/mL标准品储备母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

用移液管将300 µL标准品稀释液（1×）移入每个管子中。使用标准品储备母液制备以下稀释系列（如下图所示）。在下次转移之前，将每个管子彻底混合。每管须充分混匀后再移液到下一管。2500 ng/mL稀释液作为标准曲线的最高点。标准品稀释液（1×）作为标准品零点（0 ng/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀请充分混合

VII.操作步骤

使用前，将所有其他试剂和样品带至室温。建议对所有标准品和样品进行复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 向每个孔中加入200 µL的检测液RD1-113。
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 µL。用封板膜封住反应孔，**用水平振荡器（0.12” 轨道），转速：500±50 rpm，室温孵育2小时**。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；

   注：*转移标准品和样品应在15分钟内完成。*
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 µL，然后将板内洗涤液吸去。重复操作2次，共洗3次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
6. 在每个微孔内加入200 µL人ApoB酶标检测抗体。用封板膜封住反应孔，**用水平振荡器（0.12”轨道），转速：500±50 rpm，室温孵育2小时**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育30分钟。注意避光**；
9. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀；
10. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确；
11. 计算结果：

    将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人ApoB浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

    如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

1. Sniderman, A.D. and S.M. Marcovina (2006) Clin. Lab. Med. 26:733.
2. Law, S.W. et al. (1986) Proc. Natl. Acad. Sci. USA 83:8142.
3. Chen, S.H. et al. (1986) J. Biol. Chem. 261:12918.
4. Yang, C.Y. et al. (1986) Nature 323:738.
5. Knott, T.J. et al. (1986) Nature 323:734.
6. Cladaras, C. et al. (1986) EMBO J. 5:3495.
7. Chen, S.H. et al. (1987) Science 238:363.
8. Powell, L.M. et al. (1987) Cell 50:831.
9. Higuchi, K. et al. (1988) Proc. Natl. Acad. Sci. USA 85:1772.
10. Young, S.G. et al. (1988) Science 241:591.
11. Cardin, A.D. et al. (1984) J. Biol. Chem. 259:8522.
12. Jiang, X.C. et al. (2001) Nat. Med. 7:847.
13. Yazdanyar, A. and X.C. Jiang (2012) Hepatology 56:576.
14. Moerland, M. et al. (2008) Arterioscler. Thromb. Vasc. Biol. 28:1277.
15. Zhao, Y. et al. (2000) Mol. Biol. Cell 11:721.
16. Quintao, E.C.R. and P.M. Cazita (2010) Atherosclerosis 209:1.
17. Chen, X. et al. (2009) Nutr. Metab. 6:49.
18. Choi, S. et al. (1995) J. Biol. Chem. 270:8081.
19. Boren, J. et al. (2001) J. Biol. Chem. 276:26916.
20. Dichek, H.L. et al. (2004) J. Lipid Res. 45:551.
21. Dichek, H.L. et al. (1998) J. Biol. Chem. 273:1896.
22. Zheng, C.Y. et al. (2006) Arterioscler. Thromb. Vasc. Biol. 26:891.
23. Segrest, J.P. (2002) Curr. Diab. Rep. 2:282.
24. Proctor, S.D. et al. (2002) Curr. Opin. Lipidol. 13:461.

96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置