PRODUCT INFORMATION & MANUAL

Human Angiopoietin-like 3 (ANGPTL3) Valukine™ ELISA

Catalog Number: VAL154

For the quantitative determination of natural and recombinant human Angiopoietin-like 3 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 202310.3

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I. BACKGROUND

Human Angiopoietin-like 3 (ANGPTL3), also known as angiopoietin-5, is a secreted glycoprotein that is structurally related to the angiopoietins. Mature human ANGPTL3 contains an N-terminal coiled-coil domain, a C-terminal fibrinogen-like domain, and a linker region. Unlike other angiopoietin-like proteins, ANGPTL3 is almost exclusively expressed in the liver (1-4).

ANGPTL3 plays an important role in lipid metabolism. In humans, genome-wide association studies have shown that sequence variants in the locus containing the ANGPTL3 gene are associated with different plasma triglyceride levels (5, 6). Population-based gene sequence analysis also reveals that a loss of function mutation in the ANGPTL3 gene is commonly present in those individuals with low plasma triglyceride levels (7). ANGPTL3 can directly inhibit lipoprotein lipase (LPL), the enzyme responsible for hydrolyzing circulating triglycerides (8). In mice, abolishing ANGPTL3 gene expression leads to elevated LPL activity and hypolipidemia. When the hyperlipidemic mice are treated with a monoclonal antibody that interferes with the binding of ANGPTL3 to LPL, their serum triglyceride levels are significantly reduced (9, 10). The functional domain that mediates the interaction between ANGPTL3 and LPL has been mapped to the N-terminal region, termed specific epitope 1 (11). The linker region in ANGPTL3 is susceptible to protease cleavage. When the cleavage occurs between amino acid 221 and 224, it separates the protein into an N-terminal coiled-coil domain- containing fragment and a C-terminal fibrinogen-like domain-containing fragment. Compared to the full-length protein, the N-terminal fragment has increased bioactivity for lowering serum triglyceride (12). ANGPTL3 is also able to inhibit endothelial lipase, whose preferred substrate is high density lipoprotein (HDL). As such, ANGPTL3 may modulate serum HDL levels (13, 14). In addition to lipid metabolism, ANGPTL3 is also involved in angiogenesis. The fibrinogen-like domain of ANGPTL3 can interact with integrin αVβ3 to induce endothelial cell adhesion, migration, and neovascularization (15). Furthermore, ANGPTL3 may promote the expansion of hematopoietic stem cells (16). It has been reported that ANGPTL3 serum levels are different in patients with certain pathological conditions when compared to those in normal individuals. For instance, it is elevated in familial hypercholesterolemia, metabolic syndrome, and insulin resistance. In hemodialysis, however, it is reduced (17-20).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Angiopoietin-like 3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Angiopoietin-like 3 present is bound by the immobilized antibody. A biotinylated detection antibody specific for human Angiopoietin-like 3 is pipetted into the wells. After washing away any unbound substances, Streptavidin-HRP are pipetted into the wells. Following a wash to remove any unbound reagent, TMB substrate solution (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human Angiopoietin-like 3 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 cell culture supernate and human serum.
- 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 Reagent 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 (pg/mL)	75.5	339.5	1469.6	77.3	348.1	1523.6
Standard Deviation	2.5	15.0	44.8	3.7	19.4	96.8
CV%	3.3	4.4	3.1	4.7	5.6	6.4

B. RECOVERY

The recovery of human Angiopoietin-like 3 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 88.3 to 97.9% with an average of 92.1%.

The recovery of human Angiopoietin-like 3 spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 84.1 to 101.5% with an average of 91.3%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human Angiopoietin-like 3 is typically less than 6.2 pg/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.

D. CALIBRATION

This immunoassay is calibrated against highly purified *Sf21*-expressed recombinant human Angiopoietin-like 3 produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with

high concentrations of human Angiopoietin-like 3 and diluted with Reagent Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	96.0	91.9-100.7
1:4	95.0	88.4-97.4
1:8	95.6	91.0-98.8
1:16	90.9	77.3-98.5

F. SAMPLE VALUES

Serum - Five human serum samples were evaluated for the presence of human Angiopoietin-like 3 in this assay. All samples measured ranged from 51.18 to 142.80 ng/mL with an average of 99.23 ng/mL.

Cell Culture Supernates - TF-1 human blood leukemia cells (1×10^5 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 20ng/mL of recombinant human GM-CSF, 100U/mL penicillin, and 100µg/mL streptomycin sulfate. MCF-7 human breast cancer cells (4×10^5 cells/mL) were cultured in DMEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human Angiopoietin-like 3, and measured ranged from 0.98 to 1.54 ng/mL with an average of 1.42 ng/ mL.

G. SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

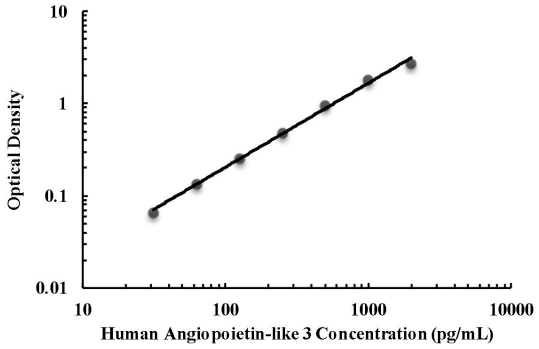
Recombinant human	Recombinant mouse
Angiopoietin-1	Angiopoietin-3
Angiopoietin-2	Angiopoietin-like 3
Angiopoietin-4	Angiopoietin-like 4
Angiopoietin-like 4	
Integrin αVβ3	

This assay also recognizes the N-terminal cleaved human Angiopoietin-like 3 (aa 17-220).

IV. EXPERIMENT

EXAMPLE STANDARD

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



pg/mL	OD	Average	Corrected
0	0.057 0.053	0.055	-
31.3	0.123 0.118	0.121	0.066
62.5	0.192 0.185	0.189	0.134
125	0.320 0.304	0.312	0.257
250	0.549 0.521	0.535	0.480
500	1.011 1.012	1.012	0.957
1000	1.905 1.849	1.877	1.822
2000	2.733 2.765	2.749	2.694

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Angiopoietin-like 3 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a rat antibody against human Angiopoietin-like 3.	1 plate
Human Angiopoietin-like 3 Standard	Recombinant human Angiopoietin-like 3 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human Angiopoietin-like 3 Detection Antibody	Biotinylated Angiopoietin-like 3 antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Detection Antibody Diluent (2×)	A 2× concentrated buffered diluent used to dilute Detection Antibody and HRP.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent (10×)	A 10× concentrated buffered protein base used to dilute standard and samples.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution	1 vial
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="8">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP A</td><td rowspan="3">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.
Standards may be stored for up to 1 month at -20°C *.</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Reagent Diluent (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody Diluent (2×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Detection Antibody 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.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.

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 protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Reagent Diluent (1×).

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 Reagent Diluent (1×).

B. SAMPLE PREPARATION

Cell Culture Supernates samples recommend at least a 10-fold dilution prior to the assay. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Reagent Diluent (1×). Optimal dilutions should be determined by the end user.

Serum samples recommend a 500-fold dilution. For example, add 10 µL of serum into a tube with 490 µL Reagent Diluent (1×) to prepare a 50-fold diluted sample. Mix through and then pipette 20 µL of prepared 50-fold diluted sample into a tube with 180 µL Reagent Diluent (1×) to prepare a final 500-fold diluted sample. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Note: *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×).

Detection Antibody Diluent (1×) - Use deionized or distilled water to prepare Detection Antibody Diluent (1×).

Reagent Diluent (1×) - Use deionized or distilled water to prepare Reagent Diluent (1×).

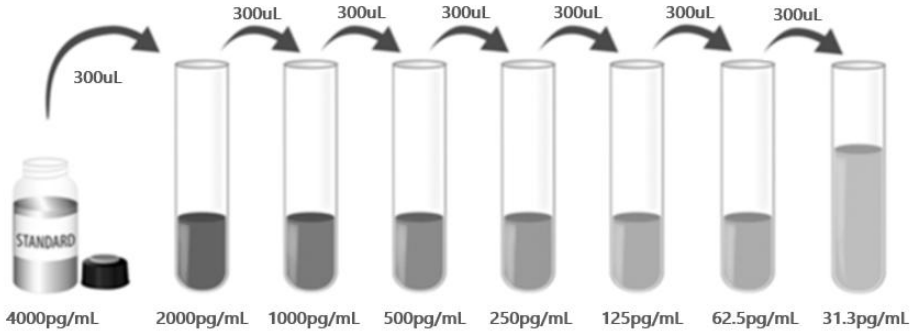
Detection Antibody (1×) - Centrifuge briefly before opening. Reconstitution volume refer to vial label to prepare Detection Antibody (100×). Allow the Detection Antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store if needed. Dilute to Detection Antibody (1×) with Detection Antibody Diluent (1×). Prepare at least 15 minutes prior to use.

Streptavidin-HRP A (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Detection Antibody Diluent (1×).

Human Angiopoietin-like 3 Standard - Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume*. This reconstitution produces a stock solution of 4000 pg/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 the appropriate Reagent Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The Reagent Diluent (1×) serves as the zero standard (0 pg/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 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 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

Note: *Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards 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 100 µL of standard, or prepared sample per well. And add 50 µL of the Detection Antibody (1×) diluted in Detection Antibody Diluent (1×), to each well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500±50 rpm.** A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Solution (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.
5. Add 100 µL of the working dilution of Streptavidin-HRP A to each well. Cover the plate and **incubate for 20 minutes at room temperature on a horizontal orbital microplate shaker set at 500±50 rpm.** Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of TMB Substrate to each well. **Incubate for 15 minutes at room temperature on a horizontal orbital microplate shaker set at 500±50 rpm.** Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. 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.

10. 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 Angiopoietin-like 3 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

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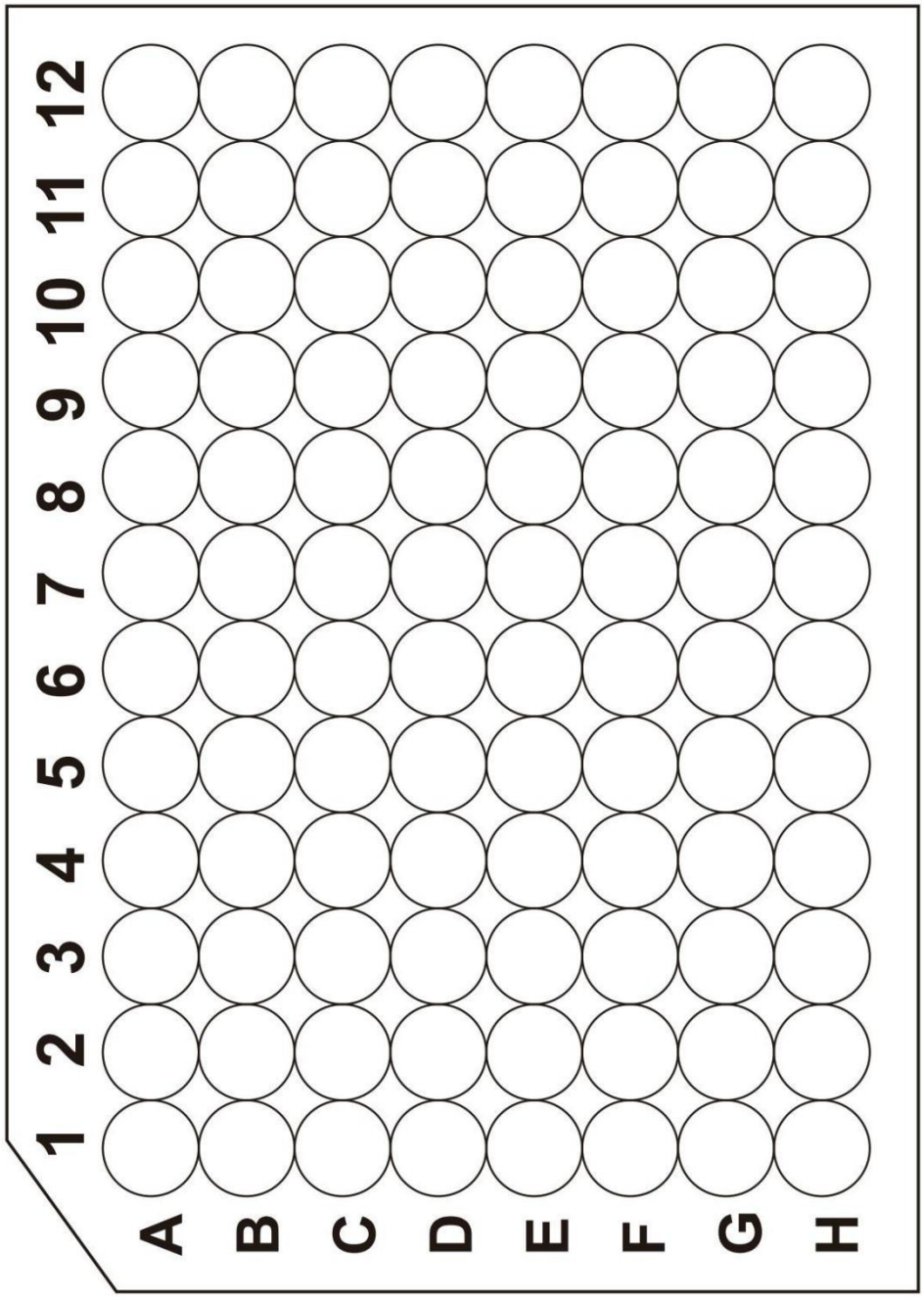
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 Angiopoietin-like 3 (ANGPTL3) Valukine™ ELISA 试剂盒

目录号：VAL154

适用于定量检测天然和重组人 Angiopoietin-like 3 的浓度

科研专用，不可用于临床诊断

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版本号 202310.3

目录

I. 背景

人血管生成素样蛋白3 (ANGPTL3)，也被称为血管生成素-5，是一种在结构上与血管生成素有关的分泌型糖蛋白。成熟的人ANGPTL3包含一个N端螺旋结构域，一个C端纤维蛋白原样结构域和一个连接区域。与其他血管生成素样蛋白不同，ANGPTL3几乎只在肝脏特定表达 (1-4)。

ANGPTL3在脂质代谢中起重要作用。在人类中，全基因组关联研究表明，包含ANGPTL3基因位点的序列，其变异情况与不同血浆的甘油三酯水平有关 (5, 6)。基于种群的基因序列分析表明，ANGPTL3基因功能缺失突变，通常发生在血浆甘油三酯水平较低的个体中 (7)。后来发现，ANGPTL3可以直接抑制脂蛋白脂肪酶(LPL)，该酶负责水解循环中的甘油三酯 (8)，因此，ANGPTL3的存在会造成血浆甘油三酯升高。在小鼠中，敲除ANGPTL3基因表达可导致LPL活性升高和血脂降低。当高脂血症小鼠用干扰ANGPTL3与LPL结合的单克隆抗体治疗时，其血清甘油三酯水平显著降低 (9, 10)。介导ANGPTL3和LPL之间相互作用的功能域已被定位到ANGPTL3的N端区域，称为特异性表位1 (11)。ANGPTL3的连接区易被蛋白酶裂解。当在其氨基酸221和224之间发生裂解时，它会将蛋白质分离成一个含有N端螺旋状结构域的片段和一个含有C端纤维蛋白原样结构域的片段。与全长蛋白质相比，N端片段具有降低血清甘油三酯的生物活性(12)。ANGPTL3还能够抑制内皮脂肪酶，其首选底物是高密度脂蛋白(HDL)。因此，ANGPTL3可以调节血清HDL水平 (13, 14)。除脂质代谢外，ANGPTL3也参与血管生成。ANGPTL3的纤维蛋白原样结构域可与整合素αVβ3相互作用，诱导内皮细胞黏附、迁移和新血管形成 (15)。此外，ANGPTL3可能促进造血干细胞的扩增 (16)。据报道，与正常个体相比，具有某些病理状况的患者血清ANGPTL3水平是各不相同的。例如，它在家族性高胆固醇血症、代谢综合征和胰岛素抵抗中会升高。然而，在血液透析患者中却降低了 (17-20)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Angiopoietin-like 3抗体包被于微孔板上，样品和标准品中的人Angiopoietin-like 3会与固定在板上的抗体结合，接着加入生物素化的抗人Angiopoietin-like 3检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶(Streptavidin-HRP)孵育。洗涤除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合的目标蛋白成正比；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；**
- 该试剂盒适用于细胞培养上清样本和人血清样本；
- 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，应将样本用试剂稀释液（1×）稀释后重新检测；
- 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，在不同板中分别检测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 (pg/mL)	75.5	339.5	1469.6	77.3	348.1	1523.6
标准差	2.5	15.0	44.8	3.7	19.4	96.8
CV%	3.3	4.4	3.1	4.7	5.6	6.4

B. 回收率

在细胞上清样本中掺入检测范围内不同水平的人Angiopoietin-like 3，测定其回收率。回收率范围在88.3-97.9%，平均回收率在92.1%。

在人血清样本中掺入检测范围内不同水平的人Angiopoietin-like 3，测定其回收率。回收率范围在84.1-101.5%，平均回收率在91.3%。

C. 灵敏度

人Angiopoietin-like 3的最低可测剂量（MDD）一般小于6.2pg/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。

D. 校正

此ELISA试剂盒经由R&D Systems生产的*Sf21*表达的高纯度重组人Angiopoietin-like 3蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人Angiopoietin-like 3，然后用试剂稀释液（1×）将样

本稀释到检测范围内，测定其线性。

稀释倍数	平均值/期待值 (%)	范围 (%)
1:2	96.0	91.9-100.7
1:4	95.0	88.4-97.4
1:8	95.6	91.0-98.8
1:16	90.9	77.3-98.5

F. 样本预值

血清样本 - 使用本试剂盒检测了5份人血清样本中人Angiopoietin-like 3的水平。5份检测值在51.18-142.80 ng/mL之间，平均值为99.23 ng/mL。

细胞上清样本 - TF-1人血白血病细胞（1×10^5细胞/mL）培养于含有10%胎牛血清、2 mM L-谷氨酰胺、20 ng/mL重组人GM-CSF、100 U/mL青霉素和100 μg/mL硫酸链霉素的RPMI 1640培养基中；MCF-7人乳腺癌细胞（4×10^5细胞/mL）培养于含有10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和100 μg/mL硫酸链霉素的DMEM培养基中。取细胞培养上清液，测定人细胞培养上清样本中人Angiopoietin-like 3的水平，检测值在0.98-1.54 ng/mL之间，平均值为1.42 ng/mL。

G. 特异性

将以下因子配制成50 ng/mL的浓度来检测没有观察到明显的交叉反应或干扰。

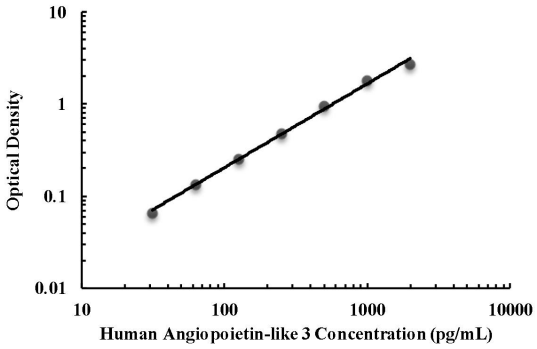
Recombinant human	**Recombinant mouse**
Angiopoietin-1	Angiopoietin-3
Angiopoietin-2	Angiopoietin-like 3
Angiopoietin-4	Angiopoietin-like 4
Angiopoietin-like 4	
Integrin αVβ3	

检测可识别N端剪切的人Angiopoietin-3 (aa 17-220)。

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。



pg/mL	OD	Average	Corrected
0	0.057 0.053	0.055	-
31.3	0.123 0.118	0.121	0.066
62.5	0.192 0.185	0.189	0.134
125	0.320 0.304	0.312	0.257
250	0.549 0.521	0.535	0.480
500	1.011 1.012	1.012	0.957
1000	1.905 1.849	1.877	1.822
2000	2.733 2.765	2.749	2.694

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Angiopoietin-like 3 Microplate	包被大鼠抗人 Angiopoietin-like 3 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human Angiopoietin-like 3 Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human Angiopoietin-like 3 Detection antibody	生物素化的 Angiopoietin-like 3 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent (10×)	浓缩的试剂稀释液（10×）用于稀释标准品和样本	1 瓶
Detection antibody Diluent (2×)	浓缩的检测抗体稀释液（2×）用于稀释检测抗体和 HRP	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	TMB ELISA 底物溶液	1 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℃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>链霉亲和素-HRP A</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*
标准品-20℃储存，最多 30 天*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</td></tr>
<tr><td>检测抗体稀释液（2×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的（1×）检测抗体稀释液，多余的丢弃</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的（1×）试剂稀释液，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℃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
- 500 mL量筒
- 振荡器（速度可调至500±50 rpm）

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。使用后请彻底洗手。

VI. 实验前准备

A. 样品收集及储存

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于-20℃冰箱内，避免反复冻融。样本可能需要用试剂稀释液（1×）稀释。

血清样本: 用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000 × g离心15分钟。吸取血清样本之后即刻用于检测，或者分装，-20℃贮存备用。避免反复冻融。样本可能需要用试剂稀释液（1×）稀释。

B. 样本准备工作

细胞上清样本建议用试剂稀释液（1×）10倍稀释后进行检测，取20 µL样本加到180 µL试剂稀释液（1×）中，充分混匀，即制备成10倍稀释的样本。最佳稀释度应由最终用户确定。

血清样本建议用试剂稀释液（1×）500倍稀释后进行检测，例如：10 µL血清加到490 µL试剂稀释液（1×）中，充分混匀，即50倍稀释。然后取20 µL 50倍稀释后的样本加到180 µL试剂稀释液（1×）中，充分混匀，即制备成500倍稀释的样本。最佳稀释度应由最终用户确定。

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20mL浓缩洗涤液用蒸馏水或去离子水稀释配制成 500mL工作浓度的洗涤液（1×）。

检测抗体稀释液（1×）：使用蒸馏水或去离子水稀释配制成检测抗体稀释液（1×）。

试剂稀释液（1×）：使用蒸馏水或去离子水稀释配制成试剂稀释液（1×）。

检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震摇至少15分钟，其充分溶解。如有需要分装保存。用检测抗体稀释液（1×）稀释至检测抗体（1×），至少在使用前15分钟准备。

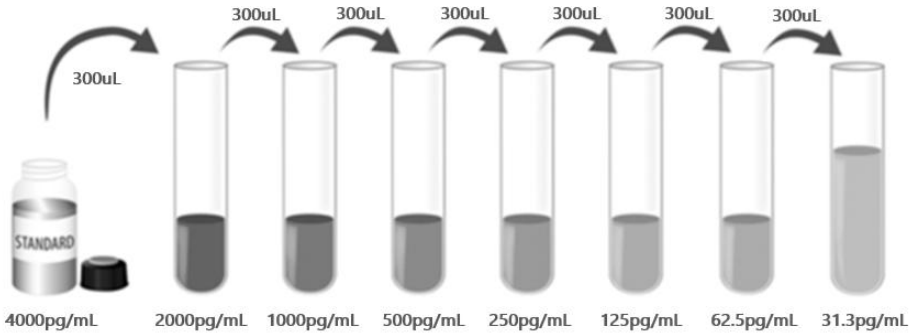
链霉亲和素- HRP A（1×）：开盖前请瞬时离心。用检测抗体稀释液（1×）将链霉亲和素

- HRP A（200×）稀释至工作浓度。

人Angiopoietin-like 3标准品：开盖前请瞬时离心。冻干标准品的重溶体积请参考瓶身标签，得到浓度为4000 pg/mL标准品母液。轻轻震摇至少15分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

向各稀释管中加入300μL试剂稀释液（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。2000 pg/mL管作标准曲线最高点，试剂稀释液（1×）可用作标准品零点（0 pg/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

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测

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. 分别将不同浓度标准品，实验样本加入相应孔中，每孔100 µL。然后每个微孔内加入50 µL配制好的检测抗体（1×），用封板膜封住反应孔，**室温500±50 rpm水平振荡孵育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；（样本需要稀释，详情参见样本制备部分。）
4. 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3次，共洗4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；
5. 在每个微孔内加入100 µL稀释好的链霉亲和素- HRP A工作液。用封板膜封住反应孔，**室温500±50 rpm水平振荡孵育20分钟，注意避光；**
6. 重复第4步洗板操作；
7. 在每个微孔内加入100 µL TMB底物溶液，**室温500±50 rpm水平振荡孵育15分钟，注意避光；**
8. 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；
9. 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；
10. **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Angiopoietin-like 3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

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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