PRODUCT INFORMATION & MANUAL

Human Angiopoietin-2 Valukine™ ELISA

Catalog Number: VAL204

For the quantitative determination of natural and recombinant human Angiopoietin-2 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

Angiopoietin-2 (Ang2) is an approximately 70 kDa secreted glycoprotein that plays a complex role in angiogenesis, inflammation, and vascular development. Angiopoietin-2 contains a coiled-coil domain that mediates multimerization and a C-terminal Fibrinogen-like domain that mediates receptor binding (1, 2). It forms disulfide-linked dimers, trimers, tetramers, and pentamers (2-4). Mature human Angiopoietin-2 shares 86% amino acid (aa) sequence identity with mouse and rat Angiopoietin-2. Angiopoietin-2 is expressed in vascular endothelial (EC) and smooth muscle cells in highly angiogenic tissues (e.g. placenta, ovaries, uterus, and tumor vasculature) (5-9), lung epithelial cells, differentiating myotubes, and neural progenitor cells (10-12). It is upregulated by cellular stress (7, 10, 13, 14) and circulates at elevated levels in sepsis and acute lung injury (10, 15).

Both Angiopoietin-2 and the related Angiopoietin-1 (Ang-1) are ligands for the receptor tyrosine kinase Tie-2 (5). Angiopoietin-2 acts as a Tie-2 agonist, although it can also partially antagonize the activation of Tie-2 by Angiopoietin-1 (5, 7, 15-17). Angiopoietin-2 promotes EC survival, proliferation, and migration, and also promotes sprouting angiogenesis (7, 17, 18). It induces the loss of pericytes from vessel walls and an increase of vascular permeability (14, 15, 19-21). Angiopoietin-2 is required for the development of lymphatic vessels as well as the postnatal remodeling of both lymphatic and vascular vessels (6, 19, 22). In cancer, Angiopoietin-2 production is induced by tumor-derived VEGF and augments VEGF-induced angiogenesis and tumor growth (8, 19, 23). In addition, Angiopoietin-2 modulates cell adhesion and promotes tumor cell migration through interactions with Integrins containing α3, α5, αV, β1, or β3 chains (24-26). It promotes leukocyte adhesion to the vascular endothelium and extravasation to inflammatory sites (10, 13, 19, 27). Aside from the circulatory system, Angiopoietin-2 promotes the differentiation of myoblasts, regulatory T cells, and neurons (11, 12, 28).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Angiopoietin-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Angiopoietin-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human Angiopoietin-2 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 Angiopoietin-2 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 supernates, human serum, human plasma and human saliva.
- 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 RD5-5 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 forty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	237	703	1301	276	805	1494
Standard Deviation	16.4	45.6	54.4	28.8	73.0	111
CV%	6.9	6.5	4.2	10.4	9.1	7.4

B. RECOVERY

The recovery of human Angiopoietin-2 spiked to different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=4)	103	98-113
Human serum* (n=4)	100	90-107
Human EDTA plasma* (n=4)	95	85-104
Human heparin plasma* (n=4)	94	87-104

*Samples were diluted prior to assay as direct in the Sample Preparation section.

C. SENSITIVITY

Thirty-eight assays were evaluated and the minimum detectable dose (MDD) of human Angiopoietin-2 ranged from 1.20-21.3 pg/mL. The mean MDD was 8.29 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 a highly purified NS0-expressed recombinant human Angiopoietin-2 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-2 and diluted with Calibrator Diluent RD5-5 to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=4)	Human serum* (n=4)	Human EDTA plasma* (n=4)	Human heparin plasma* (n=4)	Human saliva (n=4)
1:2	Average % of Expected	100	95	97	95	105
	Range (%)	98-102	87-103	93-103	89-101	101-110
1:4	Average % of Expected	97	95	95	94	107
	Range (%)	90-103	86-112	93-98	88-101	100-112
1:8	Average % of Expected	96	95	100	91	107
	Range (%)	87-105	85-114	89-113	87-100	104-112

*Samples were diluted prior to assay as directed in the Sample Preparation section.

F. SAMPLE VALUES

Human Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Angiopoietin-2 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Human serum (n=60)	2494	1065-8907	1341
Human EDTA plasma (n=35)	1964	1071-4389	808
Human heparin plasma (n=35)	2049	1009-4973	913

Human saliva - Nine samples were evaluated for the presence of human Angiopoietin-2 in this assay and ranged from 247-822 pg/mL.

Cell Culture Supernates - Two cultures of HUVEC human umbilical vein endothelial cells were grown to confluency in EGM supplemented with 2% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate and bovine brain extract. Aliquots of the cell culture supernates were removed and assayed for levels of human Angiopoietin-2.

Stimulant	**Values (pg/mL)**
HUVEC Culture 1	2326
HUVEC Culture 2	7611

Note: *Significant levels of Angiopoietin-2 are found in fetal bovine, bovine, porcine, equine, and rabbit serum. The background level of Angiopoietin-2 in control medium should be determined and subtracted from samples of conditioned medium*.

G. SPECIFICITY

This assay recognizes natural and recombinant human Angiopoietin-2.

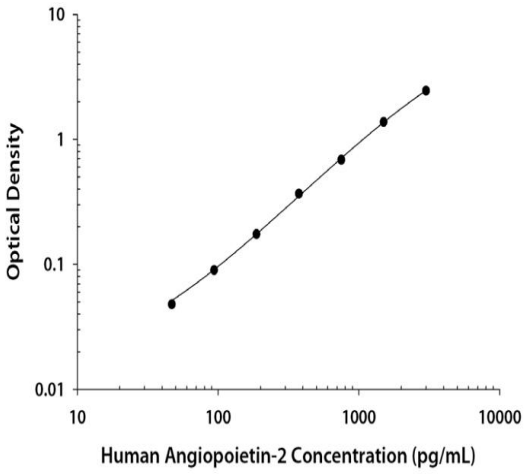
The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-5 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Angiopoietin-2 standard were also assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human：	
ANG	$VEGF_{121}$
Ang-1	$VEGF_{165}$
Tie-2	VEGF/PlGF

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)	O.D.	Average	Corrected
0	0.026 0.027	0.027	—
46.9	0.074 0.076	0.075	0.048
93.7	0.114 0.120	0.117	0.090
187.5	0.197 0.206	0.202	0.175
375	0.391 0.398	0.395	0.368
750	0.701 0.728	0.715	0.688
1500	1.394 1.418	1.406	1.379
3000	2.454 2.506	2.480	2.453

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Angiopoietin-2 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Angiopoietin-2	1 plate
Human Angiopoietin-2 Conjugate	Solution of antibody against human Angiopoietin-2 conjugated to horseradish peroxidase	1 vial
Human Angiopoietin-2 Standard	Recombinant human Angiopoietin-2 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	2 vials
Assay Diluent RD1-76	A buffered protein base	1 vial
Calibrator Diluent RD5-5	A 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="5">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>Assay Diluent RD1-76</td></tr>
<tr><td>Calibrator Diluent RD5-5</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">Use a fresh standard for each assay. Discard after use.</td></tr>
<tr><td>Standard</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 (0.12” orbit) capable of maintaining a speed of 500 ± 50 rpm
- Collection device for saliva samples that has no protein binding or filtering capabilities such as a Salivette® or equivalent
- Polypropylene test tubes for dilution of standards and samples.

D. PRECAUTION

- Angiopoietin-2 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.
- 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.

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.

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 Calibrator Diluent RD5-5.

***Caution*:** *Significant levels of Angiopoietin-2 are found in fetal bovine, bovine, porcine, equine, and rabbit sera. The background level of Angiopoietin-2 in control medium should be determined and subtracted from samples of conditioned medium.*

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 RD5-5.

Plasma - Collect plasma using EDTA or heparin 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 RD5-5.

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

Saliva - Collect saliva using a collection device such as a Salivette® or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-5.

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

B. SAMPLE PREPARATION

Use polypropylene tubes.

Human serum and plasma samples recommend a 5-fold dilution. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD5-5. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of Angiopoietin-2 are found in saliva. We recommend using a face mask and gloves to protect kit reagents from contamination.*

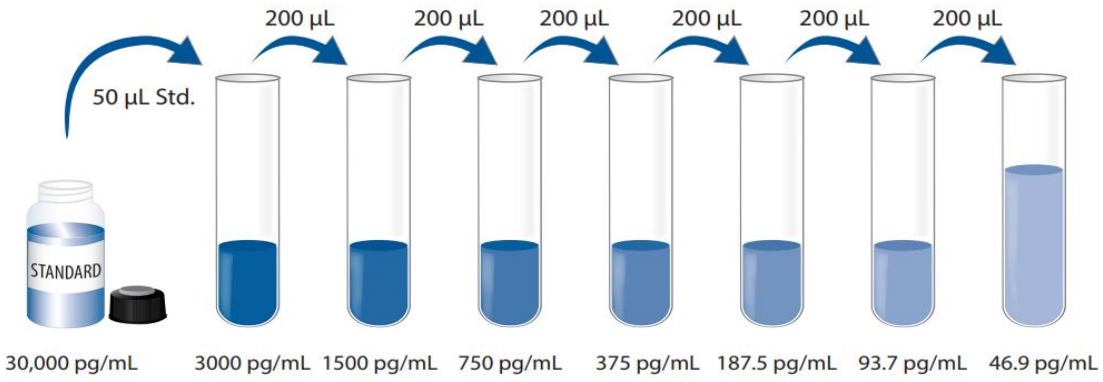
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL

of Wash Buffer (1×).

Human Angiopoietin-2 Standard - Refer to the vial label for the reconstitution volume* Reconstitute the Human Angiopoietin-2 Standard with deionized or distilled water . This reconstitution produces a stock solution of 30000 pg/mL. Mix the standard to ensure complete reconstitution and 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.

Use polypropylene tubes. Pipette 450 µL of the Calibrator Diluent RD5-5 into the 3000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 3000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent RD5-5 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 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.

NOTE: *Angiopoietin-2 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.*

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 100 µL of Assay Diluent RD1-76 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 microplate shaker (0.12" orbit) set at 500 rpm ± 50 rpm.** A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four 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 Angiopoietin-2 Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 rpm ± 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 on the benchtop. 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 Angiopoietin-2 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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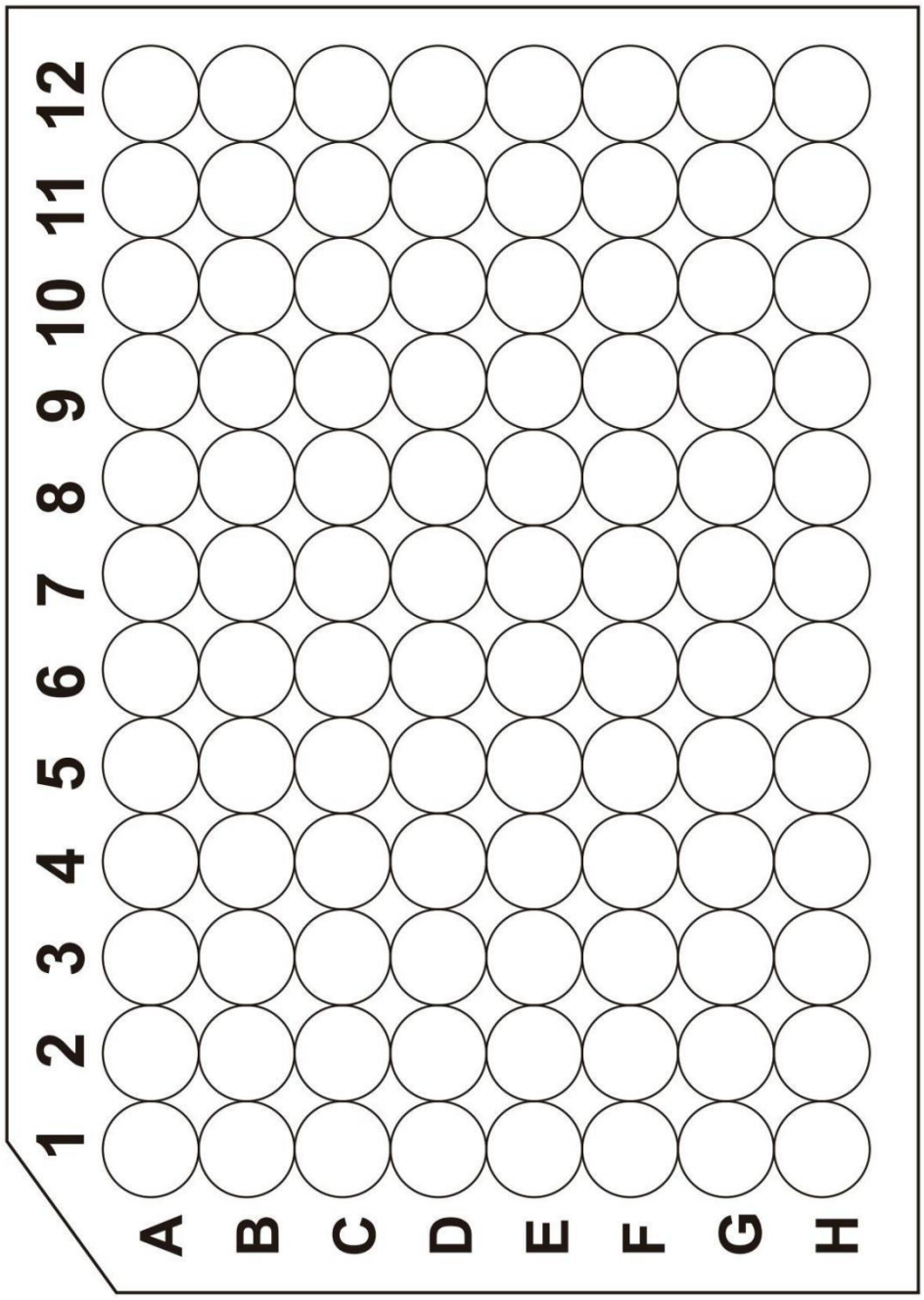
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 Angiopoietin-2 Valukine™ ELISA 试剂盒

目录号：VAL204

适用于定量检测天然和重组人血管生成素 2（Angiopoietin-2）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

血管生成素-2（Angiopoietin-2，Ang2）是一种约 70 kDa 的分泌型糖蛋白，在血管生成、炎症和血管发育中发挥着复杂的作用。血管生成素-2 包含一个介导多聚化的卷曲螺线结构域和一个介导受体结合的 C 末端纤维蛋白原样结构域（1,2）。它可形成二硫键连接的二聚体、三聚体、四聚体和五聚体（2-4）。成熟的人血管生成素-2 与小鼠和大鼠血管生成素-2 有 86%的氨基酸（aa）序列相同性。血管生成素-2 表达于高血管生成组织（如胎盘、卵巢、子宫和肿瘤血管）中的血管内皮细胞（vascular endothelial，EC）和平滑肌细胞（5-9）、肺上皮细胞、分化的肌管和神经祖细胞（10-12）。它在细胞应激时上调（7,10,13,14），并在败血症和急性肺损伤时以升高的水平循环（10,15）。

血管生成素-2和相关的血管生成素-1（Angiopoietin-1，Ang-1）都是受体酪氨酸激酶Tie-2的配体（5）。血管生成素-2是Tie-2的激动剂，但它也能部分拮抗血管生成素-1对Tie-2的激活作用（5,7,15-17）。血管生成素-2能促进EC的存活、增殖和迁移，还能促进血管萌芽生成（7,17,18）。它可诱导血管壁上的周细胞脱落，并增加血管的通透性（14,15,19-21）。淋巴管的发育以及出生后淋巴管和血管的重塑都需要血管生成素-2（6,19,22）。在癌症中，血管生成素-2的产生是由肿瘤来源的VEGF诱导的，并能促进VEGF诱导的血管生成和肿瘤生长（8,19,23）。此外，血管生成素-2通过与含有α3、α5、αV、β1或β3链的整合素相互作用，调节细胞粘附性并促进肿瘤细胞迁移（24-26）。它能促进白细胞粘附到血管内皮，并向炎症部位外渗（10,13,19,27）。除循环系统外，血管生成素-2还能促进成肌细胞、调节性T细胞和神经元的分化（11,12,28）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Angiopoietin-2抗体包被于微孔板上，样品和标准品中的人Angiopoietin-2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Angiopoietin-2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；**
- 该试剂盒适用于细胞培养上清样本，人血清样本、人血浆样本和人唾液样本；
- 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，应将样本用标准品稀释液RD5-5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，在不同板间分别检测40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)	237	703	1301	276	805	1494
标准差	16.4	45.6	54.4	28.8	73.0	111
CV%	6.9	6.5	4.2	10.4	9.1	7.4

B. 回收率

不同类型样本中掺入检测范围内不同水平的人Angiopoietin-2，测定其回收率。

样本类型	平均回收率 (%)	范围 (%)
细胞培养基 (n=4)	103	98-113
人血清样本* (n=4)	100	90-107
人 EDTA 血浆样本* (n=4)	95	85-104
人肝素血浆样本* (n=4)	94	87-104

*根据样品制备部分的说明，在检测前对样品进行稀释。

C. 灵敏度

38次试验评估表明，人Angiopoietin-2的最低检测剂量(MDD)范围为1.20-21.3 pg/mL。平均MDD为8.29 pg/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

该免疫测定法以R&D Systems生产的高纯度的NS0表达的重组人Angiopoietin-2校正。

E. 线性

不同的样本中含有或掺入高浓度的人Angiopoietin-2，然后用标准品稀释液RD5-5将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养基 (n=4)	人血清* (n=4)	人EDTA血浆* (n=4)	人肝素血浆* (n=4)	人唾液* (n=4)
1:2	平均值/期待值 (%)	100	95	97	95	105
	范围 (%)	98-102	87-103	93-103	89-101	101-110
1:4	平均值/期待值 (%)	97	95	95	94	107
	范围 (%)	90-103	86-112	93-98	88-101	100-112
1:8	平均值/期待值 (%)	96	95	100	91	107
	范围 (%)	87-105	85-114	89-113	87-100	104-112

*根据样品制备部分的指示，在检测前稀释样品。

F. 样本预值

人血清/血浆-使用此试剂盒评估健康志愿者样本中 Angiopoietin-2 的存在。本研究中使用的供体没有病史。

样本类型	平均值 (pg/mL)	范围 (pg/mL)	标准偏差(pg/mL)
人血清 (n=60)	2494	1065-8907	1341
人 EDTA 血浆 (n=35)	1964	1071-4389	808
人肝素血浆 (n=35)	2049	1009-4973	913

人唾液-使用此试剂盒评估了 9 份样本中 Angiopoietin-2 的存在，其含量范围为 247-822 pg/mL。

细胞培养上清：

HUVEC 人脐静脉内皮细胞培养在两种含2% 胎牛血清、2 mM L-谷氨酰胺、100 U/mL 青霉素、100 µg/mL 硫酸链霉素和牛脑提取物的EGM培养基中。取出等量的细胞培养上清，检测人Angiopoietin-2的水平。

刺激类型	**含量 (pg/mL)**
细胞培养1	2326
细胞培养2	7611

注意：*胎牛、牛、猪、马和兔血清中的 Angiopoietin-2 含量较高。应确定对照培养基中血管生成素-2 的背景水平，并从条件培养基样本中减去。*

G. 特异性

此ELISA法可检测天然及重组人Angiopoietin-2。

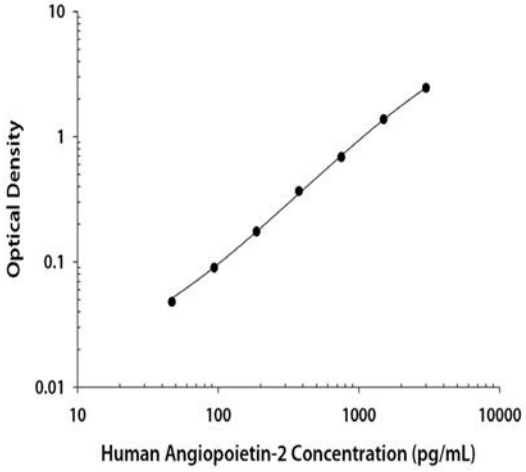
将以下因子用标准品稀释液RD5-5配制成50 ng/mL的浓度来检测与人Angiopoietin-2的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组人Angiopoietin-2标准品中，来检测对人Angiopoietin-2的干扰。没有观察到明显的交叉反应或干扰。

Recombinant human：	
ANG	$VEGF_{121}$
Ang-1	$VEGF_{165}$
Tie-2	VEGF/PlGF

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)	O.D.	Average	Corrected
0	0.026 0.027	0.027	—
46.9	0.074 0.076	0.075	0.048
93.7	0.114 0.120	0.117	0.090
187.5	0.197 0.206	0.202	0.175
375	0.391 0.398	0.395	0.368
750	0.701 0.728	0.715	0.688
1500	1.394 1.418	1.406	1.379
3000	2.454 2.506	2.480	2.453

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Angiopoietin-2 Microplate	包被抗人Angiopoietin-2抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human Angiopoietin-2 Conjugate	酶标检测抗人Angiopoietin-2抗体	1瓶
Human Angiopoietin-2 Standard	人Angiopoietin-2标准品（冻干），参考瓶身标签进行重溶	2瓶
Assay Diluent RD1-76	检测液	1瓶
Calibrator Diluent RD5-5	标准品稀释液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℃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="6">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>检测液RD1-76</td></tr>
<tr><td>标准品稀释液RD5-5</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>每次检测都使用新的标准品。使用后丢弃</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
- 500 mL量筒
- 水平振荡器（0.12”轨道），转速：500±50 rpm
- 唾液样本收集设备，不具备蛋白质结合或过滤功能，如 Salivette® 或类似设备
- 标准品及样品稀释用聚丙烯管

D. 注意事项

- Angiopoietin-2 可在唾液中检测到。在进行本检测时，请采取预防措施防止试剂盒试剂受到污染。
- 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。

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。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液RD5-5稀释。

注意： *胎牛、牛、猪、马和兔血清中含有大量Angiopoietin-2。应确定对照培养基中Angiopoietin-2 的背景水平，并从条件培养基样本中减去。*

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 RD5-5 进行稀释。

血浆 - 使用EDTA或肝素作为抗凝剂收集血浆。然后1000 × g离心15分钟。需在30分钟内收集血浆样本之后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RD5-5 稀释。

注意： *枸橼酸钠血浆尚未被验证用于该试验。*

唾液 - 使用Salivette®等设备收集唾液。立即测定或分装并储存样品在≤-20℃。避免反复冻融循环。样品可能需要用标准品稀释液RD5-5稀释。

注意： *唾液收集器不得具有任何蛋白质结合或过滤功能。*

B. 样品制备

使用聚丙烯管。

人血清和血浆样本建议稀释 5 倍。建议的 5 倍稀释是 50 µL 样品 + 200 µL 标准品稀释液 RD5-5。最佳稀释倍数应由最终用户确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

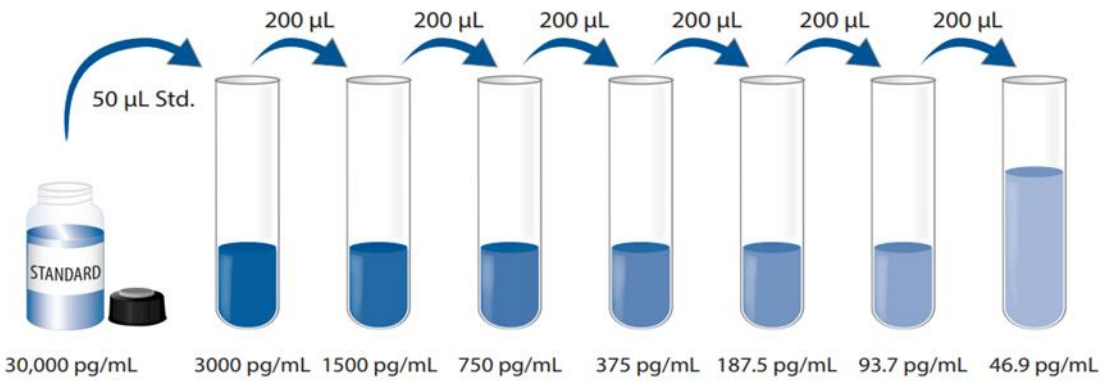
注意： *唾液中含有高浓度的Angiopoietin-2。我们建议使用口罩和手套来保护试剂盒试剂免受污染。*

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×）。

人Angiopoietin-2标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水重溶人Angiopoietin-2标准品，得到浓度为30000 pg/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。

使用聚丙烯管。将450 µL标准品稀释液RD5-5移入3000 pg/mL的管中。剩余每管中加入200 µL标准品稀释液RD5-5。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。3000 pg/mL作标准曲线最高点，标准品稀释液RD5-5移入可用作标准曲线零点（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，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。

注意：*唾液中含有高浓度的Angiopoietin-2。测试时采取预防措施，保护试剂盒试剂免受污染*

1. 按照上一节的说明，准备好所有需要的试剂，标准品和样本；
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100 µL检测液RD1-76。
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，**在水平振荡器（0.12”轨道）转速：500±50 rpm上，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；
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；
6. 在每个微孔内加入200 µL人Angiopoietin-2酶标检测抗体。用封板膜封住反应孔，**在水平振荡器（0.12”轨道）转速：500±50 rpm上，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Angiopoietin-2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