PRODUCT INFORMATION & MANUAL

Mouse VEGF Valukine™ ELISA

Catalog Number: VAL608

For the quantitative determination of natural and recombinant mouse VEGF 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 202609.6

TABLE OF CONTENTS

I. BACKGROUND 2

II. OVERVIEW 4

III. ADVANTAGES 5

IV. EXPERIMENT 9

V. KIT COMPONENTS AND STORAGE 10

VI. PREPARATION 13

VII. ASSAY PROCEDURE 15

VIII. REFRERENCES 17

I. BACKGROUND

Vascular endothelial growth factor (VEGF or VEGF-A), also known as vascular permeability factor (VPF), is a potent mediator of both angiogenesis and vasculogenesis in the fetus and adults (1-3). It is a member of the PDGF family that is characterized by the presence of eight conserved cysteine residues in a cystine knot structure and the formation of anti-parallel disulfide-linked dimers (4). Alternately spliced isoforms of 120, 164 and 188 amino acids (aa) have been found in mouse, while 121, 145, 165, 183, 189, and 206 aa isoforms have been identified in human (2, 4). In humans, $VEGF_{165}$ appears to be the most abundant and potent isoform, followed by $VEGF_{121}$ and $VEGF_{189}$ (3, 4). The same pattern may exist in mouse. Isoforms other than $VEGF_{120}$ and $_{121}$ contain basic heparin-binding regions and are not freely diffusible (4). Mouse $VEGF_{164}$ shares 97% aa sequence identity with corresponding regions of rat VEGF. It also shares 89% aa identity with human and porcine VEGF, 88% with bovine VEGF, and 90% with feline, equine and canine VEGF. VEGF is expressed in multiple cells and tissues including skeletal and cardiac muscle (5, 6), hepatocytes (7), osteoblasts (8), neutrophils (9), macrophages (10), keratinocytes (11), brown adipose tissue (12), $CD34^+$ stem cells (13), endothelial cells (14), fibroblasts, and vascular smooth muscle cells (15). VEGF expression is induced by hypoxia and cytokines such as IL-1, IL-6, IL-8, Oncostatin M and TNF-α (3, 4, 9, 16). The isoforms are differentially expressed during development and in the adult (3).

VEGF dimers bind to two related receptor tyrosine kinases, VEGF R1 (also called Flt-1) and VEGF R2 (Flk-1/KDR) and induce their homodimerization and autophosphorylation (3, 4, 7, 17, 18). These receptors have seven extracellular immunoglobulin-like domains and an intracellular split tyrosine kinase domain. They are expressed on vascular endothelial cells and a range of non-endothelial cells. Although VEGF affinity is highest for binding to VEGF R1, VEGF R2 appears to be the primary mediator of VEGF angiogenic activity (3, 4). $VEGF_{165}$ also binds the semaphorin receptor, neuropilin-1, which promotes complex formation with VEGF R2 (19).

VEGF is best known for its role in vasculogenesis. During embryogenesis, VEGF regulates the proliferation, migration, and survival of endothelial cells (3, 4), thus regulating blood vessel density and size but playing no role in determining vascular patterns. VEGF promotes bone formation through osteoblast and chondroblast

recruitment and is also a monocyte chemoattractant (20-22). In postnatal life, VEGF maintains endothelial cell integrity and is a potent mitogen for micro- and macro-vascular endothelial cells. In adults, VEGF functions mainly in wound healing and the female reproductive cycle (3). In diseased tissues, VEGF promotes vascular permeability. It is thus thought to contribute to tumor metastasis by promoting both extravasation and tumor angiogenesis (23, 24). Various strategies have been employed therapeutically to antagonize VEGF-mediated tumor angiogenesis (25). Circulating VEGF levels correlate with disease activity in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus (26).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse VEGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse VEGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse VEGF 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 mouse VEGF 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, tissue homogenates,mouse serum and mouse 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 RD5T 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)	37.7	144	277	38.3	138	291
Standard Deviation	3.1	6.2	12.9	3.2	7.8	18.5
CV%	8.2	4.3	4.7	8.4	5.7	6.4

B. RECOVERY

The recovery of mouse VEGF spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture supernates (n=9)	103	95-114
Tissue homogenates (n=3)	91	76-107
Serum* (n=9)	101	83-115
EDTA plasma* (n=5)	91	82-103
Heparin plasma* (n=4)	89	87-94

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

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse VEGF is typically less than 3 pg/mL.

D. CALIBRATION

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.

This immunoassay is calibrated against highly purified *Sf21*-expressed recombinant mouse $VEGF_{164}$ produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of mouse VEGF and Calibrator Diluent RD5T to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=2)	**Tissue homogenates (n=2)**	**Serum* (n=4)**	**EDTA plasma* (n=4)**	**Heparin plasma* (n=4)**
1:2	Average % of Expected	101	106	101	101	113
	Range (%)	93-109	101-113	95-108	98-102	111-115
1:4	Average % of Expected	98	113	104	107	115
	Range (%)	92-103	105-124	87-120	98-112	112-117
1:8	Average % of Expected	96	115	106	105	112
	Range (%)	86-101	105-117	89-115	94-112	109-118
1:16	Average % of Expected	97	118	98	101	95
	Range (%)	81-106	103-130	90-106	85-120	82-119

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

F. SAMPLE VALUES

Serum/Plasma - Forty individual mouse serum samples and nine individual mouse plasma samples were evaluated for detectable levels of mouse VEGF in this assay. All samples measured less than the lowest mouse VEGF standard, 7.8 pg/mL.

Tissue Homogenates - Tissue homogenates from mouse heart, lung, kidney, and spleen tissue were assayed for mouse VEGF and measured 111 pg/mL, 3680 pg/mL, 157 pg/mL, and 30 pg/mL, respectively.

Cell Culture Supernates:

P388D1 mouse lymphoma cells (1 x 10^5 cells/mL) were cultured for 4 days in RPMI supplemented with 5% fetal bovine serum. The cell culture supernate was assayed for mouse VEGF and measured 7600 pg/mL.

Mouse lung conditioned media (1 lung, 1-2 mm pieces in 10 mL of RPMI supplemented with 10% fetal bovine serum) was collected after culturing for 5 days. The cell culture supernate was assayed for mouse VEGF and measured 4660 pg/mL.

Mouse heart conditioned media (1 heart, 1-2 mm pieces in 10 mL of RPMI supplemented with 10% fetal bovine serum) was collected after culturing for 5 days. The cell culture supernate was assayed for mouse VEGF and measured 378 pg/mL.

G. SPECIFICITY

This assay recognizes both the 164 and 120 amino acid residue forms of mouse VEGF. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5T and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse VEGF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse		Recombinant human	Natural proteins
C10	L-13	Flt-3 Ligand	human PDGF
G-CSF	KC	PlGF	
GM-CSF	JE/MCP-1	PDGF-AA	
IFN-γ	LIF	PDGF-AB	
IL-1α	M-CSF		
IL-1β	MIP-1α		
IL-2	MIP-1β		
IL-3	MIP-2		
IL-4	PlGF-2		
IL-5	SCF		
IL-6	TNF-α		
IL-7	Tpo		
IL-9	VEGF-B		
IL-10	VEGF-D		
IL-10 sR	VEGF R2		
IL-12	VEGF R3		

Some cross-reactivity was observed with the following:

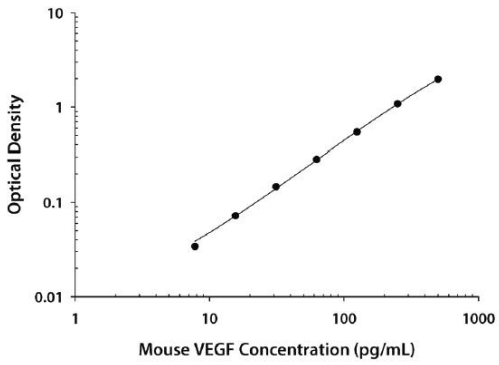
Factor	Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-Reactivity
$rhVEGF_{165}$	50,000	106	0.2
$rhVEGF_{165}$/PlGF	50,000	27	0.05
rrVEGF	1000	250	25
rm $VEGF_{188}$	5000	457	9

Recombinant mouse VEGF R1 interferes in this assay at concentrations > 2500 pg/mL.

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	0.061 0.070	0.066	—
7.8	0.099 0.102	0.100	0.034
15.6	0.139 0.136	0.138	0.072
31.3	0.213 0.211	0.212	0.146
62.5	0.358 0.342	0.350	0.284
125	0.624 0.600	0.612	0.546
250	1.141 1.156	1.148	1.082
500	1.969 2.103	2.036	1.970

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse VEGF Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against mouse VEGF	1 plate
Mouse VEGF Conjugate	Solution of an antibody against mouse VEGF conjugated to horseradish peroxidase with preservatives	1 vial
Mouse VEGF Standard	Recombinant mouse VEGF in a buffered protein base with preservatives; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Assay Diluent RD1N	A buffered protein base with preservatives. May contain a precipitate. Mix well before and during use.	1 vial
Calibrator Diluent RD5T	A 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/TMB Substrate Solution	1 vial
Stop Solution	Diluted hydrochloric acid solution	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="6">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Assay Diluent RD1N</td></tr>
<tr><td>Calibrator Diluent RD5T</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at ≤-20°C in a manual defrost freezer. * Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the oil ouch 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.
- Polypropylene test tubes for dilution of standards and samples

D. PRECAUTION

- Assay Diluent RD1N contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.
- The Stop Solution provided with this kit is an acid solution.
- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- TMB substrate may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
- Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling

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.

Tissue Homogenates - The preparation of tissue homogenates will vary depending upon the tissue type. For this assay, heart, kidney, and spleen tissue from three mice and lung tissue from eight mice were rinsed with 1× PBS to remove excess blood, homogenized in 20 mL of 1× PBS, and stored overnight at ≤ -20°C.

After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 × g. Samples can be assayed immediately or stored at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 × 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 20 minutes at 2000 × 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.*

B. SAMPLE PREPARATION

Use polypropylene tubes. Serum and plasma samples require a 5-fold dilution into Calibrator Diluent RD5T. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD5T.

Cell culture supernates and tissue homogenates may require dilution. The dilution factor is dependent upon sample values. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Note: Bring all reagents to room temperature before use.

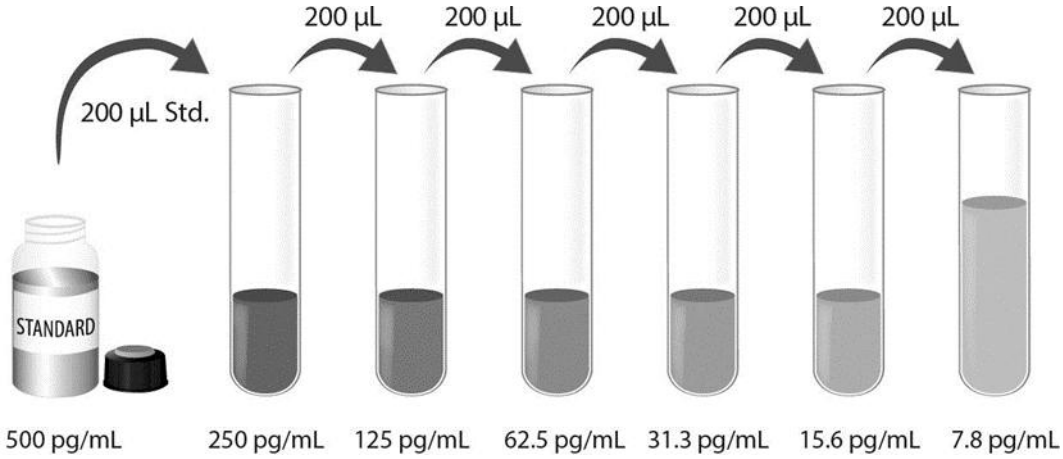
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL

of Wash Buffer (1×).

Mouse VEGF Standard - Refer to the vial label for reconstitution volume*. Reconstitute the Mouse VEGF Standard with Calibrator Diluent RD5T. Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

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

Use polypropylene tubes . Pipette 200 µL of Calibrator Diluent RD5T into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard 500 pg/mL serves as the high standard. The Calibrator Diluent RD5T 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

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 ,samples 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 50 µL of Assay Diluent RD1N to each well.
4. Add 50 µL of standard or sample per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.** A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five 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 100 µL of mouse VEGF conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature**. **Protect from light**.
9. Add 100 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 mouse VEGF 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.

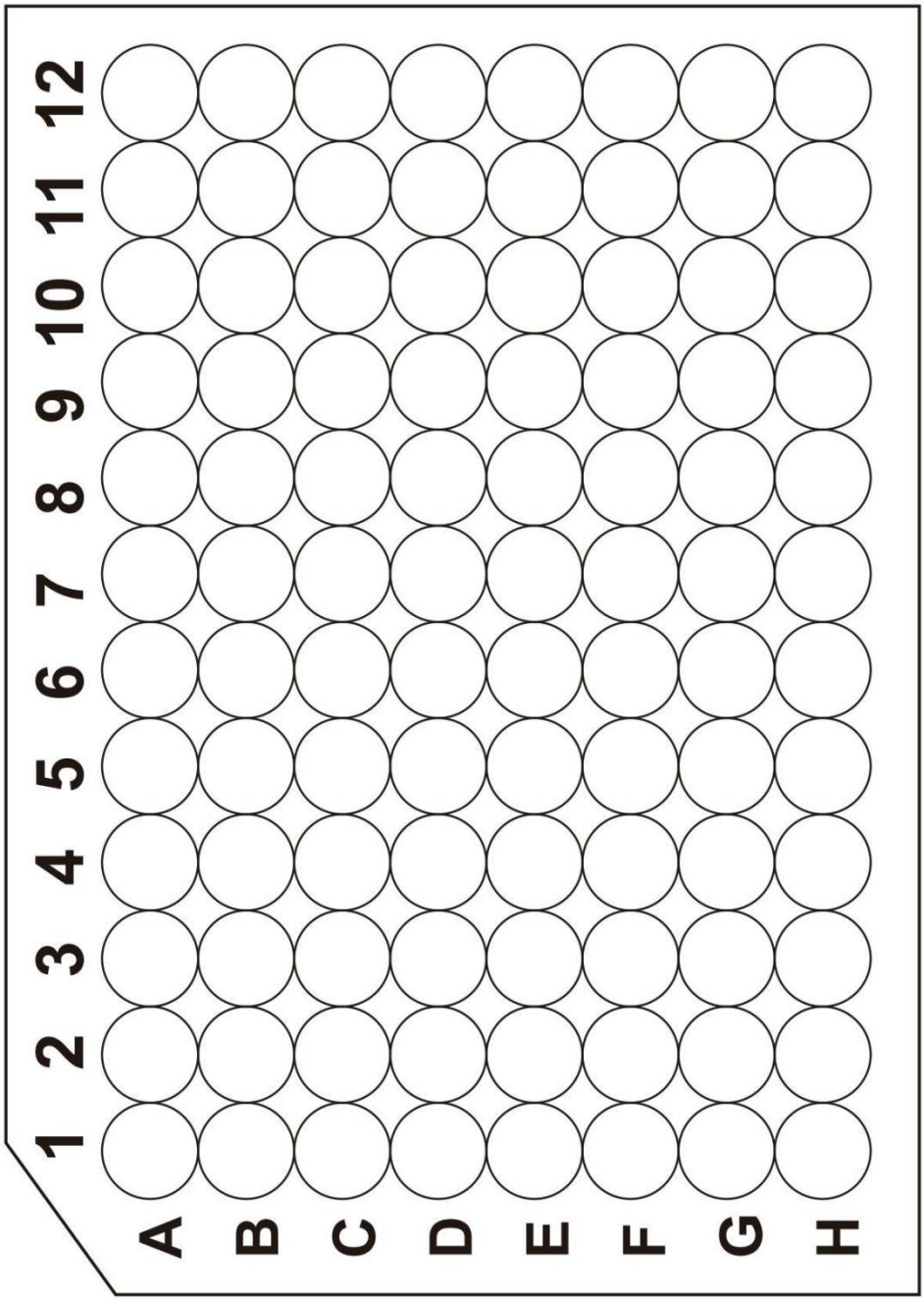
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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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

小鼠 VEGF Valukine™ ELISA 试剂盒

目录号：VAL608

适用于定量检测天然和重组小鼠 VEGF 的浓度

科研专用，不可用于临床诊断

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版本号 202609.6

目录

I. 背景 **21**

II. 概述 **22**

III. 优势 **23**

IV. 实验 **27**

V. 试剂盒组成及储存 **28**

VI. 实验前准备 **30**

VII. 操作步骤 **32**

VIII. 参考文献 **33**

I. 背景

血管内皮生长因子(VEGF 或 VEGF-A)，又叫血管通透因子(VPF)，是一种针对胎儿和成人的血管新生和血管发生的有效调节因子(1-3)。血管内皮生长因子属于 PDGF 家族，该家族蛋白的特点是存在 8 个保守的半胱氨酸结点和通过反向平行二硫键结合的二聚体 (4)。经过选择性剪切，VEGF 剪接体有不同的氨基酸长度。小鼠有 $VEGF_{120}$、$VEGF_{164}$、$VEGF_{188}$；人的 VEGF 有 $VEGF_{121}$、$VEGF_{145}$、$VEGF_{165}$、$VEGF_{183}$、$VEGF_{189}$ 和 $VEGF_{206}$ (2, 4)。$VEGF_{165}$ 是人的表达量最高、活性最强的一种亚型，其次是 $VEGF_{121}$ 和 $VEGF_{189}$ (3, 4)。同样的模式也可能存在于小鼠中。除 $VEGF_{120}$ 和 $VEGF_{121}$ 外，VEGF 的其他亚型都含有基本的肝素结合区域，且不能自由扩散(4)。小鼠 $VEGF_{164}$ 与相应的大鼠蛋白的氨基酸序列享有 97%的同源性，与人或猪的 VEGF 同源性为 89%，与牛 VEGF 同源性为 88%，与猫、马及犬的 VEGF 的同源性为 90%。VEGF 在多种细胞和组织中表达，包括骨骼肌和心肌细胞(5, 6)、肝细胞(7)、成骨细胞(8)、中性粒细胞(9)、巨噬细胞(10)、角质形成细胞(11)、棕色脂肪细胞(12)、CD34+干细胞(13)、内皮细胞(14)、成纤维素细胞、血管平滑肌细胞(15)等。VEGF 的表达受到缺氧和细胞因子的诱导，包括 IL-1、IL-6、IL-8、抑瘤素 M (Oncostatin M)和肿瘤坏死因子α等(3, 4, 9, 16)。在发育过程和成体中，VEGF 亚型的表达也是不同的(3)。

VEGF 的二聚体与两个相关的酪氨酸激酶受体相结合，即 VEGF R1 (也叫 FLt-1)和 VEGF R2 (Flk-1/KDR)。VEGF 与受体的结合可诱导后者同型二聚体化和自磷酸化(3, 4, 7, 17, 18)。这些受体拥有 7 个胞外的类免疫球蛋白域。血管内皮细胞和一些非内皮细胞都有 VEGF 受体的表达。尽管 VEGF 与 VEGF R1 的亲和力最高，但 VEGF R2 却是调控 VEGF 的血管新生活性的主要因子(3, 4)。$VEGF_{165}$ 也结合臂板蛋白(semaphoring)受体、神经纤毛蛋白 1 (neuropilin-1)，由此促进与 VEGF R2 的复合体形成(19)。

VEGF 因其参与血管发生而著称。在胚胎发育过程中，VEGF 调控内皮细胞的增殖、迁移和生存(3, 4)，并由此调控血管的密度、体积，但对于血管形成的格局并不起作用。VEGF 通过成骨细胞和软骨细胞的招募促进骨骼的形成，它同时也是一个单核细胞趋化因子 (20-22)。在产后，VEGF 维持血管内皮细胞的完整性，并且是大/微血管内皮细胞的有效的有丝分裂剂。在成体中，VEGF 主要在伤口修复和女性的生殖周期发挥作用(3)。在疾病组织中，VEGF 促进血管的通透性。因此，VEGF 通过促进外渗和肿瘤血管生成，参与了肿瘤的转移过程(23, 24)。针对阻断 VEGF 活性的各种治疗策略正在被用于控制由 VEGF 诱导的肿瘤血管生成(25)。体内循环的 VEGF 水平与自身免疫行疾病（如类风湿关节炎、多发性硬化症、系统性红斑狼疮）的病征程度相关(26)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠VEGF抗体包被于微孔板上，样品和标准品中的小鼠VEGF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小鼠VEGF抗体；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，组织匀浆，小鼠血清和小鼠血浆样本；
- 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RD5T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)	37.7	144	277	38.3	138	291
标准差	3.1	6.2	12.9	3.2	7.8	18.5
CV%	8.2	4.3	4.7	8.4	5.7	6.4

B. 回收率

在不同样本中掺入检测范围内三个水平的小鼠VEGF，测定其回收率。

样本类型	平均回收率（%）	范围（%）
细胞培养上清(n=9)	103	95-114
组织匀浆液 (n=3)	91	76-107
血清* (n=9)	101	83-115
EDTA血浆* (n=5)	91	82-103
肝素血浆*(n=4)	89	87-94

*样本在检测前已按照样本准备部分的说明进行了稀释。

C. 灵敏度

小鼠VEGF的最低可测值一般小于3 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

此ELISA试剂盒经R&D Systems生产的*Sf21*表达的高纯度重组小鼠$VEGF_{164}$蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠VEGF，然后用标准品稀释液RD5T将样本稀释到检测范围内，测定其线性。

		细胞培养上清 (n=2)	组织匀浆液 (n=2)	血清* (n=4)	EDTA 血浆*(n=4)	肝素血浆* (n=4)
1:2	平均值/期待值（%）	101	106	101	101	113
	范围 (%)	93-109	101-113	95-108	98-102	111-115
1:4	平均值/期待值（%）	98	113	104	107	115
	范围 (%)	92-103	105-124	87-120	98-112	112-117
1:8	平均值/期待值（%）	96	115	106	105	112
	范围 (%)	86-101	105-117	89-115	94-112	109-118
1:16	平均值/期待值（%）	97	118	98	101	95
	范围 (%)	81-106	103-130	90-106	85-120	82-119

*样本在检测前已按照样本准备部分的说明进行了稀释。

F. 样本预值

血清/血浆-在本检测中，对40份单个小鼠血清样本和9份单个小鼠血浆样本进行了可检测水平的小鼠VEGF评估。所有样本的测量值均低于小鼠VEGF最低标准品浓度7.8 pg/mL。

组织匀浆——对来自小鼠心脏、肺、肾脏和脾脏的组织匀浆进行小鼠VEGF检测，测得结果分别为111 pg/mL、3680 pg/mL、157 pg/mL和30 pg/mL。

细胞培养上清：

P388D1小鼠淋巴瘤细胞（1×10^5 个细胞/mL）在含5%胎牛血清的RPMI培养基中培养4天。对细胞培养上清液进行小鼠VEGF检测，测得结果为7600 pg/mL。

小鼠肺条件培养基（1个肺，切成1-2毫米小块，置于10 mL含10%胎牛血清的RPMI培养基中）在培养5天后收集。对细胞培养上清液进行小鼠VEGF检测，测得结果为4660 pg/mL。

小鼠心脏条件培养基（1个心脏，切成1-2毫米小块，置于10 mL含10%胎牛血清的RPMI培养基中）在培养5天后收集。对细胞培养上清液进行小鼠VEGF检测，测得结果为378 pg/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠VEGF蛋白。将以下因子用标准品稀释液RD5T配制成50 ng/mL的浓度来检测与小鼠VEGF的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组小鼠VEGF对照品中，来检测对小鼠VEGF的干扰。没有观察到明显的交叉反应或干扰。

Recombinant mouse		**Recombinant human**	**Natural proteins**
C10	L-13	Flt-3 Ligand	human PDGF
G-CSF	KC	PlGF	
GM-CSF	JE/MCP-1	PDGF-AA	
IFN-γ	LIF	PDGF-AB	
IL-1α	M-CSF		
IL-1β	MIP-1α		
IL-2	MIP-1β		
IL-3	MIP-2		
IL-4	PlGF-2		
IL-5	SCF		
IL-6	TNF-α		
IL-7	Tpo		
IL-9	VEGF-B		
IL-10	VEGF-D		
IL-10 sR	VEGF R2		
IL-12	VEGF R3		

以下因子存在一定的交叉反应：

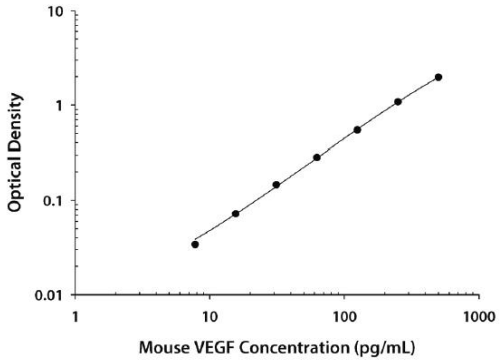
因子	检测浓度 (pg/mL)	实测值 (pg/mL)	交叉反应性 (%)
$rhVEGF_{165}$	50,000	106	0.2
$rhVEGF_{165}$/PlGF	50,000	27	0.05
rrVEGF	1000	250	25
rm $VEGF_{188}$	5000	457	9

在该检测中，重组小鼠VEGF R1在浓度高于2500 pg/mL时会产生干扰。

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	0.061 0.070	0.066	—
7.8	0.099 0.102	0.100	0.034
15.6	0.139 0.136	0.138	0.072
31.3	0.213 0.211	0.212	0.146
62.5	0.358 0.342	0.350	0.284
125	0.624 0.600	0.612	0.546
250	1.141 1.156	1.148	1.082
500	1.969 2.103	2.036	1.970

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse VEGF Microplate	包被抗小鼠 VEGF 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse VEGF Conjugate	酶标抗小鼠 VEGF 检测抗体	1 瓶
Mouse VEGF Standard	小鼠 VEGF 标准品（冻干粉），溶于含防腐剂的缓冲蛋白基质中，参考瓶身标签进行重溶	1 瓶
Assay Diluent RD1N	含防腐剂的缓冲蛋白基质。	1 瓶
Calibrator Diluent RD5T	含防腐剂的缓冲蛋白基质。用于稀释样本和标准品	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	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="7">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="5">2-8℃储存，最多 30 天*。</td></tr>
<tr><td>终止液</td></tr>
<tr><td>检测液 RD1N</td></tr>
<tr><td>标准品稀释液 RD5T</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB 底物溶液</td><td rowspan="2">分装，-20 ℃以下冰箱储存 30 天*；避免反复冻融。</td></tr>
<tr><td>标准品</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；
- 用于稀释标准品和样本的聚丙烯试管。

D. 注意事项

- 检测液RD1N 含有叠氮化钠，叠氮化钠可能与铅管和铜管发生反应，生成具有爆炸性的金属叠氮化物。请用大量水冲洗处理。
- 本试剂盒提供的终止液为酸性溶液。
- 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。
- TMB底物溶液可能造成皮肤，眼睛和呼吸道刺激，避免吸入。
- 穿戴防护手套、防护服、眼罩和面罩。操作后彻底清洗双手。

VI. 实验前准备

A. 样品收集及储存

以下样本采集和储存条件仅供一般性参考。样本稳定性尚未经过评估。

细胞培养上清液样本：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°C冰箱内，避免反复冻融。

组织匀浆样本：组织匀浆的制备方法因组织类型而异。在本检测中，取三只小鼠的心脏、肾脏和脾脏组织以及八只小鼠的肺组织，用1×PBS冲洗以去除多余血液，在20 mL 1× PBS中匀浆，并于≤ -20°C条件下冷冻过夜。

经过两次冻融循环以破碎细胞膜后，将匀浆液在5000 × g条件下离心5分钟。样品可立即进行检测，或在≤ -20°C条件下保存。避免反复冻融。

血清样本：将血液样本在室温下静置凝集2小时，然后在2000 × g条件下离心20分钟。取出血清后立即检测，或分装后在≤ -20°C条件下保存。避免反复冻融。

血浆样本：使用EDTA或肝素作为抗凝剂采集血浆。采集后30分钟内，在2000 × g条件下离心20分钟。立即检测，或分装后在≤ -20°C条件下保存。避免反复冻融。

注意：*枸橼酸盐血浆尚未经验证可用于本检测。*

B. 样本准备工作

使用聚丙烯试管。血清和血浆样本需用校准品稀释液RD5T进行5倍稀释。建议的5倍稀释为：50 µL样本 + 200 µL校准品稀释液RD5T。

细胞培养上清样品和组织匀浆样本可能需要稀释，稀释倍数取决于样本测定值。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。

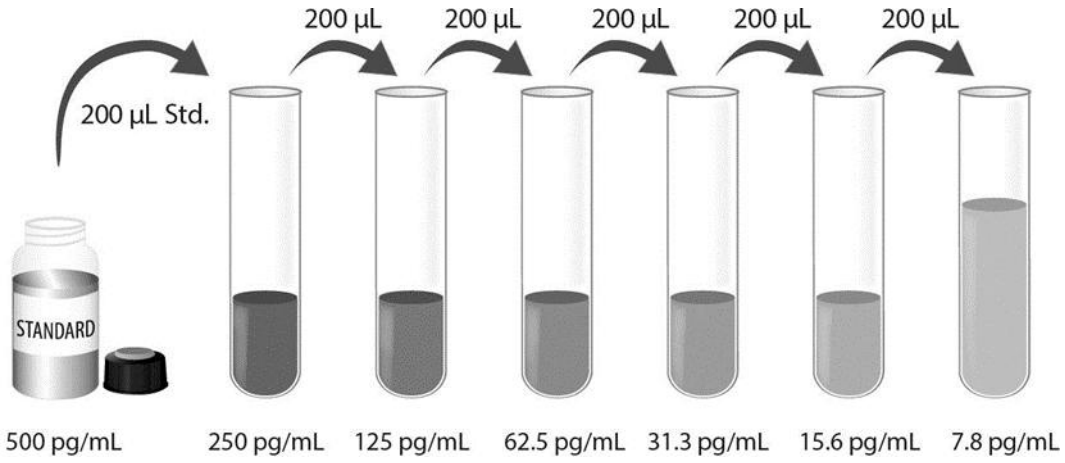
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

小鼠VEGF标准品：参照标准品瓶身注明的方式重溶冻干标准品*。使用标准品稀释液

RD5T重溶小鼠VEGF标准品。请勿使用其他稀释液。得到浓度为500 pg/mL标准品母液。轻轻震摇至少5分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

使用聚丙烯试管。向各稀释管中加入200 µL标准品稀释液RD5T。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。没有稀释的标准品母液可用作标准曲线最高点（500 pg/mL），标准品稀释液RD5T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，样本和标准品；
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. 在每个微孔中加入50 µL检测液RD1N；
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。轻轻敲击板框1分钟以混合均匀。用封板膜封住反应孔，**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4次，共洗5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100 µL酶标检测抗体。用封板膜封住反应孔，**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100 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100 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。数据可以通过绘制小鼠VEGF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

