PRODUCT INFORMATION & MANUAL

Mouse VEGF Valukine™ ELISA

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

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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 adult (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 antiparallel 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, VEGF165 appears to be the most abundant and potent isoform, followed by VEGF121 and VEGF189 (3, 4). The same pattern may exist in mouse. Isoforms other than VEGF120 and 121 contain basic heparin-binding regions and are not freely diffusible (4). Mouse VEGF164 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), keratinocy1es (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 autophosphorytation (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). VEGF165 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. Monoclonal 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 polyclonal antibody specific for mouse VEGF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of 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 and mouse 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 Calibrator Diluent 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)

Two 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)	27.5	107.7	289.7	28.0	103.5	298.2
Standard Deviation	2.2	5.0	13.4	2.3	6.1	21.4
CV%	7.9	4.6	4.6	8.0	5.9	7.2

B. RECOVERY

The recovery of mouse VEGF spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 99.6-118.4% with an average of 108.9%.

The recovery of mouse VEGF spiked to four levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 77.9-90.0% with an average of 84.2%.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse VEGF is typically less than 2.64 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 mouse VEGF 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 (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	99.6	97.0 – 108.9
1:4	103.4	95.1 – 111.7
1:8	103.5	97.6 -119.1
1:16	101.4	95.0 -115.1

F. SAMPLE VALUES

Cell Culture Supernates - 3T3 NIH swiss mice cells (1.58×10^4 cells/mL) were cultured in DMEM supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cell culture supernate was assayed for mouse VEGF and measured 372.6 pg/mL.

CTLL-2 mouse T Lymphocytes cells (2.56×10^4 cells/mL) were cultured in RPMI-1640 supplemented with 10% fetal calf serum, 100 ng/mL of recombinant mouse IL-2, 0.11 g/L Sodium pyruvate, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cell culture supernate was assayed for mouse VEGF and measured 74 pg/mL.

L929 mice NCTC clone 929 cells (3.25×10^5 cells/mL) were cultured in DMEM supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cell culture supernate was assayed for mouse VEGF and measured 150.2 pg/mL.

Serum - Three mouse serum samples were evaluated for the presence of VEGF in this assay. All samples measured ranged from 60.5 to 234 pg/mL with an average of 127 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 and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse VEGF standard were assayed for interference. No significant cross-reactivity or interference was observed.

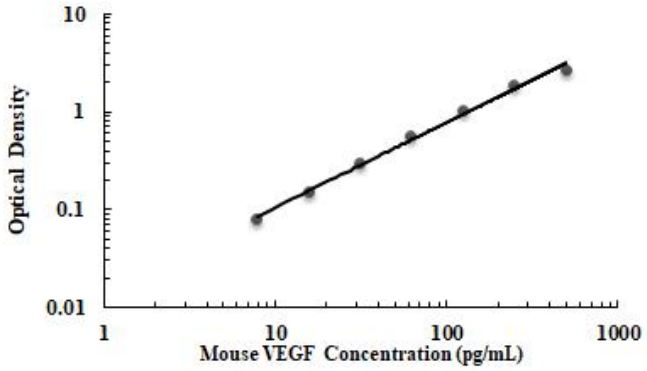
Recombinant mouse	
VEGF115	VEGF R2
VEGF167	VEGF R3
VEGF186	

At concentrations greater than 78 pg/mL, rmVEGF R1 interferes in this assay.

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	0.078 0.077	0.078	-
7.8	0.155 0.158	0.157	0.079
15.6	0.229 0.233	0.231	0.154
31.3	0.371 0.367	0.369	0.292
62.5	0.627 0.632	0.630	0.552
125.0	1.117 1.117	1.117	1.040
250.0	1.956 1.880	1.918	1.841
500.0	2.738 2.718	2.728	2.651

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 monoclonal antibody against mouse VEGF	1 plate
Mouse VEGF Conjugate	Solution of a polyclonal 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
Calibrator Diluent (2×)	Concentrated buffered diluent used to dilute standard and samples	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives	1 vial
Color Reagent A	Stabilized hydrogen peroxide	1 vial
Color Reagent B	Stabilized chromogen (tetramethylbenzidine)	1 vial
Stop Solution	Diluted hydrochloric acid solution	1 vial
Plate Covers	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>Diluted Wash Solution (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>Calibrator Diluent (1×)</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</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.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.
- 500 mL graduated cylinder.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
- 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 ℃. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator 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 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

B. SAMPLE PREPARATION

Cell Culture Supernates samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of cell culture supernates sample + 100 µL of Calibrator Diluent (1×).

Mouse serum samples require a 5-fold dilution. A suggested 5-fold dilution is 40 µL of mouse serum sample + 160 µL of Calibrator Diluent (1×).

C. REAGENT PREPARATION

Note: Bring all reagents to room temperature before use.

Wash Solution - 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.

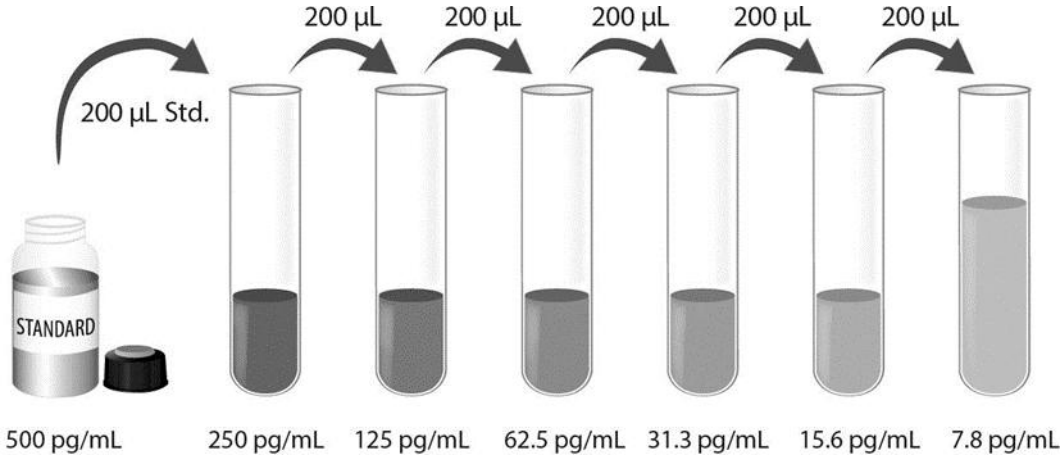
Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

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

Mouse VEGF Standard - Refer to the vial label for reconstitution volume* using Calibrator Diluent (1×). This reconstitution produces a stock solution of 500 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 200 µL of appropriate 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 undiluted standard 500 pg/mL serves as the high standard. The Calibrator 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.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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 50 µL of Calibrator Diluent (1×) to each well.
4. Add 50 µL of Standard, sample, or control per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature **on a horizontal orbital microplate shaker set at 500±50rpm.** 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 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.
6. Add 100 µL of mouse VEGF conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature **on a horizontal orbital microplate shaker set at 500±50rpm. Protect from light**.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on a horizontal orbital microplate shaker set at 500± 50rpm**. **Protect from light**.
9. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

11. **CALCULATION OF RESULTS.** Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

    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 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.

VIII. RERERENCES

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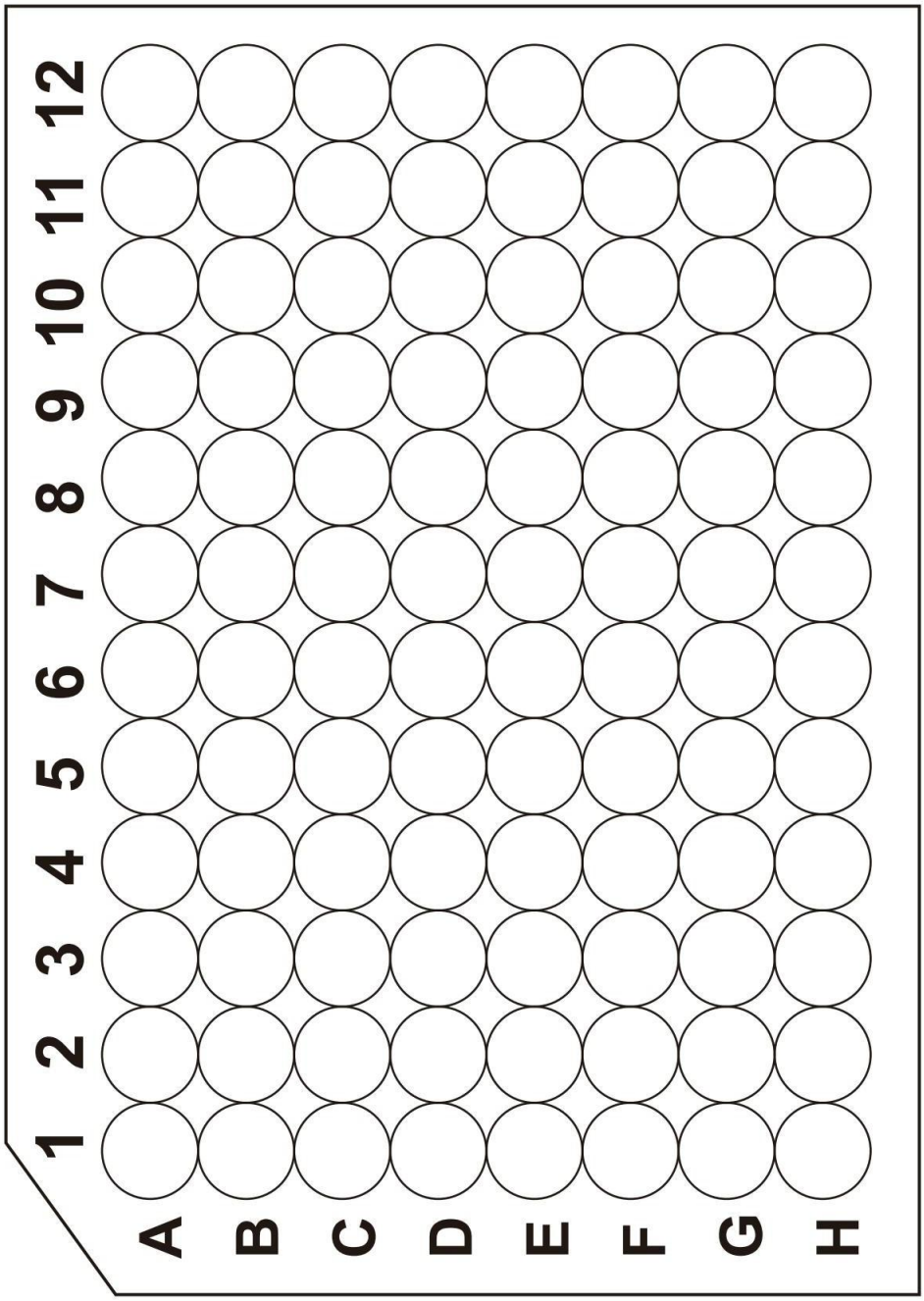
PLATE LAYOUT

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 的含量

科研专用，不可用于临床诊断

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目录

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VEGF120、VEGF164、VEGF188；人的VEGF有VEGF121、VEGF145、VEGF165、VEGF183、VEGF189和VEGF206 (2, 4)。VEGF165是人的表达量最高、活性最强的一种亚型，其次是VEGF121和VEGF89 (2, 3)。同样的模式也可能存在于小鼠中。除VEGF120和VEGF121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VEGF164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)。VEGF165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。

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单抗包被于微孔板上，样品和标准品中的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多抗，与结合在微孔板上的VEGF结合而形成免疫复合物，游离的成分被洗去；加入底物溶液（显色剂），溶液颜色逐渐变成蓝色，加入终止液溶液变黄并且停止变化。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小鼠血清样本；
- 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、试剂盒的储存等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的两个样本，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)	27.5	107.7	289.7	28.0	103.5	298.2
标准差	2.2	5.0	13.4	2.3	6.1	21.4
CV%	7.9	4.6	4.6	8.0	5.9	7.2

B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的小鼠VEGF，测定其回收率。回收率范围在99.6-118.4%， 平均回收率在108.9%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠VEGF，测定其回收率。回收率范围77.9-90.0%，平均回收率在84.2 %。

C. 灵敏度

小鼠VEGF的最低可测值一般小于2.64 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	99.6	97.0 - 108.9
1:4	103.4	95.1 - 111.7
1:8	103.5	97.6 -119.1
1:16	101.4	95.0 -115.1

F. 样本预值

细胞上清样本 - 3T3小鼠胚胎成纤维细胞（1.58×10^4细胞/mL）培养于含有10%胎牛血清的DMEM培养基中，含100U/mL青霉素，100μg/mL硫酸链霉素，取细胞上清液测VEGF含量，结果为372.6pg/mL。

CTLL-2小鼠T淋巴细胞（2.56×10^4细胞/mL）培养于含有10%胎牛血清的RPMI1640培养基中，细胞培养基还含有100 ng/mL的小鼠IL-2重组蛋白，0.11 g/L的丙酮酸钠，100 U/mL青霉素，100μg/mL硫酸链霉素，取细胞上清液测VEGF含量，结果为74pg/mL。

L929小鼠成纤维细胞（3.25×10^5细胞/mL）培养于含有10%胎牛血清的DMEM培养基中，100 U/mL青霉素，100μg/mL硫酸链霉素，取细胞上清液测VEGF含量，结果为150.2 pg/mL。

血清样本 - 使用本试剂盒检测了3份小鼠血清样本中VEGF的水平。3份样本的检测值在60.5-234 pg/mL，平均值为127pg/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠VEGF蛋白。将以下因子用稀释液（1×）配置成50ng/mL的浓度来检测与小鼠VEGF的交叉反应。将50ng/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。

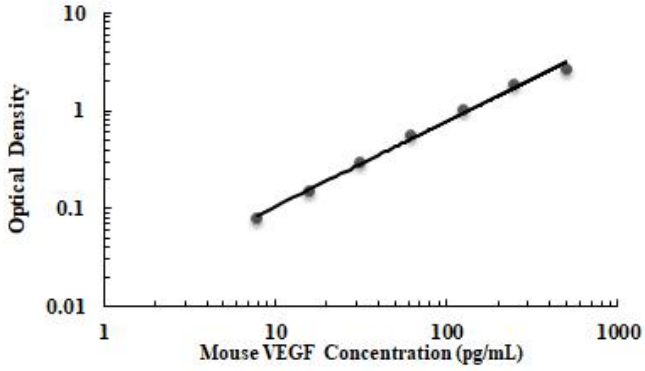
Recombinant mouse	
VEGF115	**VEGF R2**
VEGF167	**VEGF R3**
VEGF186	

当浓度大于78 pg/mL时，重组人VEGF R1在本试剂盒中有干扰作用。

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	0.078 0.077	0.078	-
7.8	0.155 0.158	0.157	0.079
15.6	0.229 0.233	0.231	0.154
31.3	0.371 0.367	0.369	0.292
62.5	0.627 0.632	0.630	0.552
125.0	1.117 1.117	1.117	1.040
250.0	1.956 1.880	1.918	1.841
500.0	2.738 2.718	2.728	2.651

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	酶标检测VEGF抗体	1瓶
Mouse VEGF Standard	标准品（冻干粉），参考瓶身标签进行重溶	1瓶
Calibrator Diluent (2×)	浓缩稀释液（2×），用于稀释标准品和样本	1瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
Color Reagent A	显色液A	1瓶
Color Reagent B	显色液B	1瓶
Stop Solution	终止液	1瓶
Plate Covers	封板胶纸	3张

* 本试剂盒包含足够的试剂以用于一块96孔微孔板的ELISA实验。

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>洗涤缓冲液（1×）</td><td rowspan="5">2-8℃储存，最多30天*。</td></tr>
<tr><td>终止液</td></tr>
<tr><td>稀释液 （1×）</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>显色液A</td></tr>
<tr><td>显色液B</td><td>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><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>
<tr><td>包被的微孔板条</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450nm检测波长的吸收值及540nm或570nm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；
- 振荡器（速度可调至500±50rpm）
- 500mL量筒；

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。
- Color Reagent B可能引起皮肤、眼睛和呼吸道刺激，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 x 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。

B. 样本准备工作

细胞上清样本需要用稀释液（1×）2倍稀释后进行检测，即100 µL细胞上清样本+100 µL稀释液（1×）。

小鼠血清样本需要用稀释液（1×）5倍稀释后进行检测，即40 µL小鼠血清样本+160 µL稀释液（1×）。

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：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置成500 mL工作浓度的洗涤液。未用完的放回4 ℃。

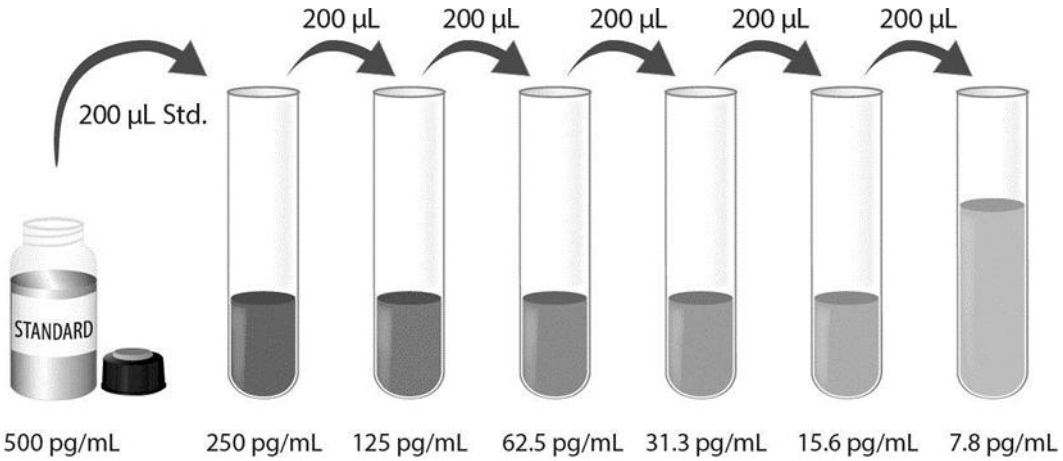
显色剂：按当次试验所需要用量将显色液A和显色液B等体积混合，避光；在使用前15分钟内准备；每孔需100µL。

稀释液（1×）：使用蒸馏水或去离子水稀释配置成稀释液（1×）。

小鼠VEGF标准品：冻干标准品的重溶用稀释液（1×）请参考瓶身标签，得到浓度为500pg/mL标准品母液。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。

向各稀释管中加入200µL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。没有稀释的标准品母液可用作标准曲线最高点（500pg/mL），稀释液（1×）可用作标准曲线零点（0pg/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，配置不同浓度标准品、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，正确使用封板胶纸可保证结果的准确性；
- 混合后的显色底物在上板前应为无色，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显色底物上板顺序一致；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. 在每个微孔中加入 50μL稀释液（1×）；
4. 分别将不同浓度标准品，实验样本或者质控品加入相应孔中，每孔50μL。用封板胶纸封住反应孔，**室温500±50rpm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；
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400μL，然后将板内洗涤液吸去。重复操作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100μL酶标检测抗体。用封板胶纸封住反应孔，**室温500±50rpm水平振荡孵育2小时，注意避光**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入100μL显色底物，**室温500±50rpm水平振荡孵育30分钟，注意避光**；
9. 在每个微孔内加入100μL终止液，孔内溶液颜色会从蓝色变为黄色。如果溶液颜色变为绿色或者颜色变化不一致，请轻拍微孔板，使溶液混合均匀；
10. 加入终止液后30分钟内，使用酶标仪测量450nm的吸光度值，设定540nm或570nm作为校正波长。如果没有使用双波长校正，结果准确度可能会受影响；
11. **计算结果：**将每个标准品和样品的校正吸光度值(OD450-OD540/OD570)、复孔读数取平均值，然后减去平均零标准品OD值。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。另一种方法是，可以通过绘制标准品浓度做对数与相应OD值对数生成曲线，并通过回归分析确定最佳拟合线。这个过程可生成一个足够使用但不太精确的数据拟合。若样本经过稀释，计算浓度时应乘以稀释倍数。

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