PRODUCT INFORMATION & MANUAL

Rat VEGF Valukine™ ELISA

Catalog Number: VAL906

For the quantitative determination of natural and recombinant rat Vascular Endothelial Growth Factor (VEGF) concentrations

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

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

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

Version 202411.1

TABLE OF CONTENTS

I. BACKGROUND 2

II. OVERVIEW 4

III. ADVANTAGES 5

IV. EXPERIMENT 10

V. KIT COMPONENTS AND STORAGE 11

VI. PREPARATION 14

VII. ASSAY PROCEDURE 16

VIII. REFERENCES 18

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 in 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 formation of anti-parallel disulfide-linked dimers (4). Alternately spliced isoforms of 120, 164, and 188 amino acids (aa) have been found in rats and mice, while 121, 145, 165, 183, 189, and 206 aa isoforms have been identified in humans (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 rats and mice. Isoforms other than $VEGF_{120}$ and $VEGF_{121}$ contain basic heparin-binding regions and are not freely diffusible (4). Rat $VEGF_{164}$ shares 97% aa sequence identity with corresponding regions of mouse, 88% with human and bovine, 89% with porcine and canine, and 90% with feline and equine 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). 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, 16, 17). 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 (18).

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 (19-21). 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 (22, 23). Various strategies have been employed therapeutically to antagonize VEGF-mediated tumor angiogenesis (24). Circulating VEGF levels correlate with disease activity in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus (25).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for rat VEGF has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any rat VEGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for rat 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 rat 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 supernates, rat serum and rat 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 RD5-18/RD5-3 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 of known concentration were tested in separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE ASSAY

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	65	60	65
Mean (pg/mL)	79.4	174	797	87.6	204	828
Standard Deviation	5.7	7.0	14.1	8.2	21.2	42.5
CV%	7.2	4.0	1.8	9.4	10.4	5.1

RAT SERUM/PLASMA ASSAY

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	71	65	71
Mean (pg/mL)	103	191	891	107	233	900
Standard Deviation	3.8	10.7	19.7	8.5	23.3	41.1
CV%	3.7	5.6	2.2	7.9	10.0	4.6

B. RECOVERY

The recovery of rat 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=6)	103	94-108
Rat Serum (n=6)	99	90-116
Rat EDTA plasma (n=6)	102	95-108
Rat Heparin plasma (n=6)	92	80-100

C. SENSITIVITY

Twenty-eight assays were evaluated and the minimum detectable dose (MDD) of rat VEGF ranged from 3.9-25.0 pg/mL. The mean MDD was 8.4 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 rat $VEGF_{164}$ produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of rat VEGF and diluted with Calibrator Diluent RD5-18/RD5-3 to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture supernates (n=6)	Rat Serum (n=6)	Rat EDTA plasma (n=6)	Rat Heparin plasma (n=6)
1:2	Average % of Expected	101	100	103	99
	Range (%)	98-104	94-103	101-106	94-102
1:4	Average % of Expected	103	101	104	102
	Range (%)	97-109	99-103	103-107	98-106
1:8	Average % of Expected	106	102	107	103
	Range (%)	99-115	97-105	102-111	96-111
1:16	Average % of Expected	105	104	115	95
	Range (%)	93-120	99-110	113-116	82-111

F. SAMPLE VALUES

Rat serum/plasma - Twelve individual serum and plasma samples were evaluated for detectable levels of rat VEGF in the assay. No detectable levels were observed.

Cell Culture Supernates :

Rat lung, cut into 1-2 mm pieces, was cultured for 4 days in 25-30 mL of RPMI supplemented with 10% fetal bovine serum and stimulated with 2.5 ng/mL LPS. The cell culture supernate was removed, assayed for rat VEGF, and measured 2264 pg/mL.

Rat spleen, cut into 1-2 mm pieces, was cultured for 3-4 days in 25-30 mL of RPMI supplemented with 10% fetal bovine serum and stimulated with 10 μg/mL ConA. The cell culture supernate was removed, assayed for rat VEGF, and measured 1105 pg/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant rat VEGF.

The factors listed below were prepared at 50 ng/mL in the appropriate Calibrator Diluent RD5-18/RD5-3 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rat VEGF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:		**Recombinant mouse:**
CINC-2α	IL-6	PIGF-2
CINC-2β	IL-10	$VEGF_{115}$
CINC-3	IL-18	$VEGF\text{-}B_{167}$
CNTF	LIX	$VEGF\text{-}B_{186}$
CNTF Rα	MAC/Fc Chimera	VEGF-D
E-Selectin/Fc Chimera	MIP-3α	VEGF R2
EphA5/Fc Chimera	β-NGF	VEGF R3
EphB1/Fc Chimera	Npn-1/Fc Chimera	**Recombinant human:**
Fractalkine	Npn-2/Fc Chimera	$VEGF_{165}$
GDNF	PDGF-AA	$VEGF\text{-}B_{167}$
GM-CSF	PDGF-AB	VEGF-C
IFN-γ	PDGF-BB	VEGF-D
IL-1α	TIMP-1	VEGF R3
IL-1β	TNF-α	**Other recombinants:**
IL-1ra		porcine PDGF
IL-2		zebrafish VEGF
IL-4		

Some cross-reactivity was observed with the following:

Recombinant Factor	**Concentration Tested (pg/mL)**	**% Cross-reactivity**
Mouse $VEGF_{120}$ (*E. coli*-expressed)	2000	59
Mouse $VEGF_{164}$ (*Sf* 21-expressed)	500	73
Canine $VEGF_{164}$ (*E. coli*-expressed)	2000	14
Human $VEGF_{121}$ (*Sf* 21-expressed)	2000	26
Human $VEGF_{121}$ (*E. coli*-expressed)	2000	31
Human $VEGF_{162}$ (NS0-expressed)	2000	24
Human $VEGF_{206}$ (*E. coli*-expressed)	50,000	0.2
Human VEGF/PlGF	10,000	5.2

Some interference was observed with the following:

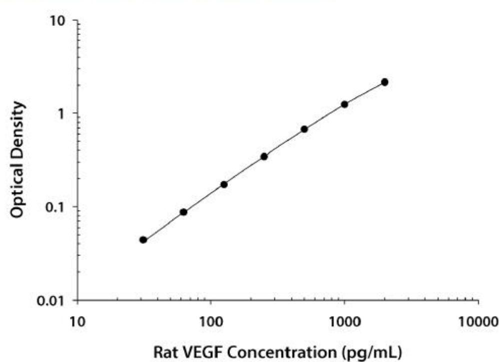
Recombinant mouse VEGF R1	At concentrations greater than 200 pg/mL
Recombinant human VEGF R1	At concentrations greater than 1500 pg/mL
Recombinant human VEGF R2	At concentrations greater than 25,000 pg/mL

IV. EXPERIMENT

EXAMPLE STANDARD

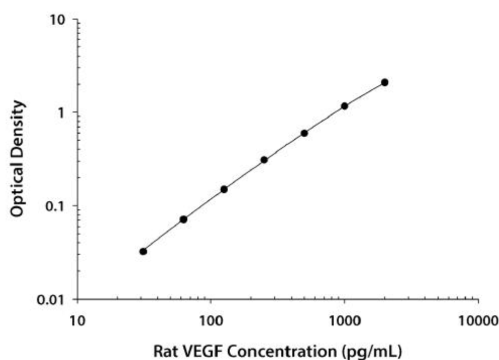
The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.118 0.119	0.119	—
31.3	0.162 0.164	0.163	0.044
62.5	0.201 0.210	0.206	0.087
125	0.287 0.293	0.290	0.171
250	0.462 0.464	0.463	0.344
500	0.791 0.795	0.793	0.674
1000	1.313 1.403	1.358	1.239
2000	2.237 2.296	2.267	2.148

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.112 0.112	0.112	—
31.3	0.140 0.147	0.144	0.032
62.5	0.179 0.187	0.183	0.071
125	0.260 0.262	0.261	0.149
250	0.415 0.426	0.421	0.309
500	0.698 0.716	0.707	0.595
1000	1.240 1.301	1.271	1.159
2000	2.128 2.266	2.197	2.085

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Rat VEGF Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against rat VEGF.	1 plate
Rat VEGF Conjugate	An antibody specific for rat VEGF conjugated to horseradish peroxidase.	1 vial
Rat VEGF Standard	Recombinant rat VEGF in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Rat VEGF Control	Recombinant rat VEGF in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	2 vial
Assay Diluent RD1-41	A buffered protein base.	1 vial
Calibrator Diluent RD5-18	A buffered surfactant used to dilute standard and samples. *For cell culture supernate samples*.	1 vial
Calibrator Diluent RD5-3	A buffered protein solution used to dilute standard and samples. *For rat serum/plasma 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.	1 vial
Stop Solution	Diluted hydrochloric 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="10">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="7">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1-41</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Control</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Calibrator Diluent RD5-18</td><td>May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Calibrator Diluent RD5-3</td><td>May be stored for up to 1 month at 2-8°C.*</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.
- Test tubes for dilution of standards and samples.
- Horizontal orbital shaker (0.12” orbit) 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 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 RD5-18.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 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 RD5-3.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 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 RD5-3.

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

Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

B. REAGENT PREPARATION

Bring all reagents to room temperature before use.

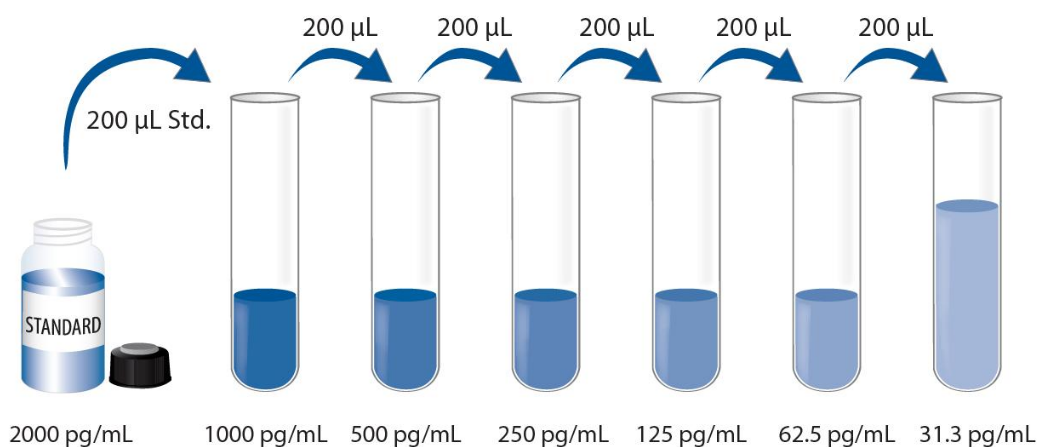
Rat VEGF Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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×).

Rat VEGF Standard- Refer to the vial label for the reconstitution volume* Reconstitute the Rat VEGF Standard with Calibrator Diluent RD5-18 (*for cell culture supernate samples*) or Calibrator Diluent RD5-3 (*for rat serum/plasma samples*). Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Allow the stock solution to sit for a minimum of 15 minutes with gentle mixing prior to making dilutions.

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

Use polypropylene tubes. Pipette 200 µL of the Calibrator Diluent RD5-18 (*for cell culture supernate samples*) or Calibrator Diluent RD5-3 (*for rat serum/plasma samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Rat VEGF Standard (2000 pg/mL) serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 pg/mL).



C. 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 reagents and samples to room temperature before use. It is recommended that all standards, samples, and control be assayed in duplicate.

1. Prepare all reagents, standard, control, 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 50 µL of Assay Diluent RD1-41 to each well.
4. Add 50 µL of standard, control and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital shaker (0.12" orbit) set at 500±50 rpm.** A plate layout is provided for a record of standards and samples assayed.
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 Rat VEGF Conjugate to each well. Cover with a new adhesive strip. **Incubate for 1 hour at room temperature on the shaker on a horizontal orbital shaker (0.12" orbit) set at 500±50 rpm.**
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, control 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 Rat 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. REFERENCES

1. Conn, G. et al. (1990) J. Biol. Chem. 87:2628.
2. Ishii, H. et al. (2001) Arch. Oral Biol. 46:77.
3. Byrne, A.M. et al. (2005) J. Cell. Mol. Med. 9:777.
4. Robinson, C.J. and S.E. Stringer (2001) J. Cell. Sci. 114:853.
5. Richardson, R.S. et al. (1999) Am. J. Physiol. 277:H2247.
6. Sugishita, Y. et al. (2000) Biochem. Biophys. Res. Commun. 268:657.
7. Yamane, A. et al. (1994) Oncogene 9:2683.
8. Goad, D.L. et al. (1996) Endocrinology 137:2262.
9. Gaudry, M. et al. (1997) Blood 90:4153.
10. Mclaren, J. et al. (1996) J. Clin. Invest. 98:482.
11. Diaz, B.V. et al. (2000) J. Biol. Chem. 275:642.
12. Asano, A. et al. (1997) Biochem. J. 328:179.
13. Bautz, F. et al. (2000) Exp. Hematol. 28:700.
14. Namiki, A. et al. (1995) J. Biol. Chem. 270:31189.
15. Nauck, M. et al. (1997) Am. J. Respir. Cell. Mol. Biol. 16:398.
16. Neufeld, G. et al. (1999) FASEB. J. 13:9.
17. Kowalewski, M.P. et al. (2005) Accession #ABB82619.
18. Pan, Q. et al. (2007) J. Biol. Chem. 282:24049.
19. Weis, S.M. and D.A. Cheresh (2005) Nature 437:497.
20. Breier, G. (2000) Semin. Thromb. Hemost. 26:553.
21. Barleon, B. et al. (1996) Blood 87:3336.
22. Weis, S.M. and D.A. Cheresh (2005) Nature 437:497.
23. Thurston, G. (2002) J. Anat. 200:575.
24. Grothey, A. and E. Galanis (2009) Nat. Rev. Clin. Oncol. 6:507.
25. Carvalho, J.F. et al. (2007) J. Clin. Immunol. 27:246.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

产品信息及操作手册

大鼠 VEGF Valukine™ ELISA 试剂盒

目录号：VAL906

适用于定量检测天然和重组大鼠血管内皮生长因子（VEGF）的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

info.cn@bio-techne.com

有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202411.1

目录

I. 背景 22

II. 概述 23

III. 优势 24

IV. 实验 28

V. 试剂盒组成及储存 29

VI. 实验前准备 31

VII. 操作步骤 33

VIII. 参考文献 34

I. 背景

血管内皮生长因子（Vascular endothelial growth factor，VEGF 或 VEGF-A），又称血管通透性因子（vascular permeability Factor，VPF），是胎儿和成人血管生成和血管形成的强效介质（1-3）。它是 PDGF 家族的一员，其特征是在胱氨酸结结构中存在八个保守的半胱氨酸残基，并形成反平行的二硫键连接的二聚体（4）。在大鼠和小鼠体内发现了 120、164 和 188 个氨基酸（amino acids，aa）的交替剪接异构体，在人类体内发现了 121、145、165、183、189 和 206 aa 的异构体（2, 4）。在人体中，$VEGF_{165}$ 是最丰富和最有效的亚型，其次是 $VEGF_{121}$ 和 $VEGF_{189}$（3, 4）。大鼠和小鼠也可能存在同样的模式。除了 $VEGF_{120}$ 和 $VEGF_{121}$ 外，其他亚型含有基本的肝素结合区，不能自由扩散（4）。大鼠 $VEGF_{164}$ 与小鼠相应区域的 aa 序列相同度为 97%，与人和牛的相同度为 88%，与猪和犬的相同度为 89%，与猫和马的 VEGF 相同度为 90%。VEGF 在多种细胞和组织中表达，包括骨骼肌和心肌（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）、棕色脂肪组织（12）、$CD34^+$ 干细胞（13）、内皮细胞（14）、成纤维细胞和血管平滑肌细胞（15）。缺氧和细胞因子（如 IL-1、IL-6、IL-8、Oncostatin M 和 TNF-α）会诱导 VEGF 的表达（3, 4, 9）。在发育过程中和成年后，这些亚型的表达有所不同（3）。

VEGF 二聚体与两种相关的受体酪氨酸激酶结合，即 VEGF R1（又称 Flt-1）和 VEGF R2（Flk-1/KDR），并诱导它们同源二聚化和自身磷酸化（3, 4, 7, 16, 17）。这些受体有七个细胞外免疫球蛋白样结构域和一个细胞内分裂酪氨酸激酶结构域。它们在血管内皮细胞和一系列非内皮细胞上表达。虽然 VEGF 与 VEGF R1 结合的亲和力最高，但 VEGF R2 似乎是 VEGF 血管生成活性的主要介质（3, 4）。$VEGF_{165}$ 还能与信号素受体 neuropilin-1 结合，从而促进与 VEGF R2 形成复合物（18）。

VEGF 因其在血管生成中的作用而最为人熟知。在胚胎发育过程中，VEGF 调节内皮细胞的增殖、迁移和存活（3, 4），从而调节血管的密度和大小，但在决定血管形态方面没有作用。VEGF 通过成骨细胞和软骨细胞的募集促进骨形成，同时也是一种单核细胞趋化诱导剂（19-21）。在出生后，VEGF 可维持内皮细胞的完整性，是微血管和大血管内皮细胞的强效有丝分裂原。在成人中，VEGF 主要在伤口愈合和女性生殖周期中发挥作用（3）。在病变组织中，VEGF 会促进血管通透性。因此，它被认为是通过促进外渗和肿瘤血管生成来促成肿瘤转移的（22, 23）。目前已采用多种治疗策略来拮抗 VEGF 介导的肿瘤血管生成（24）。循环中的 VEGF 水平与类风湿性关节炎、多发性硬化症和系统性红斑狼疮等自身免疫性疾病的病情活动相关（25）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗大鼠VEGF抗体包被于微孔板上，样品、质控品和标准品中的大鼠VEGF会与固定在板上的抗体结合，游离的成分被洗去；加入过氧化酶标记的抗大鼠VEGF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，大鼠血清样本和大鼠血浆样本；
- 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RD5-18/RD5-3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分别进行了独立检测，以评估检测间的精密度。检测由至少三名技术人员使用两种批次的试剂进行。

细胞培养上清样本检测

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
样本量	20	20	20	65	60	65
平均值（pg/mL)	79.4	174	797	87.6	204	828
标准偏差	5.7	7.0	14.1	8.2	21.2	42.5
CV%	7.2	4.0	1.8	9.4	10.4	5.1

大鼠血清/血浆样本检测

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
样本量	20	20	20	71	65	71
平均值（pg/mL)	103	191	891	107	233	900
标准偏差	3.8	10.7	19.7	8.5	23.3	41.1
CV%	3.7	5.6	2.2	7.9	10.0	4.6

B. 回收率

在不同类型样本中掺入检测范围内不同水平的大鼠VEGF，测定其回收率。

样本类型	平均回收率%	范围 (%)
细胞培养基 (n=6)	103	94-108
大鼠血清 (n=6)	99	90-116
大鼠EDTA血浆 (n=6)	102	95-108
大鼠肝素血浆 (n=6)	92	80-100

C. 灵敏度

测定28次，大鼠VEGF的最小可检测剂量（MDD）范围为3.9-25.0 pg/mL。平均MDD为8.4 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表达的重组大鼠$VEGF_{164}$校正。

E. 线性

不同的样本中含有或掺入高浓度的大鼠VEGF，然后用标准品稀释液RD5-18/RD5-3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=6)	大鼠血清 (n=6)	大鼠EDTA血浆 (n=6)	大鼠肝素血浆 (n=6)
1:2	平均值/期待值（%）	101	100	103	99
	范围（%）	98-104	94-103	101-106	94-102
1:4	平均值/期待值（%）	103	101	104	102
	范围（%）	97-109	99-103	103-107	98-106
1:8	平均值/期待值（%）	106	102	107	103
	范围（%）	99-115	97-105	102-111	96-111
1:16	平均值/期待值（%）	105	104	115	95
	范围（%）	93-120	99-110	113-116	82-111

F. 样本预值

大鼠血清/血浆样本 - 评估了12份独立的血清和血浆样品在测定中可检测到的大鼠VEGF水平。未观察到可检测水平。

细胞上清样本 - 将大鼠肺切成1-2 mm的小块，在25-30 mL补充有10%胎牛血清的RPMI中培养4天，并用2.5 ng/mL LPS刺激。收集细胞培养上清液，测定大鼠VEGF，测得浓度为2264 pg/mL。

将大鼠脾脏切成1-2 mm的小块，在25-30 mL补充有10%胎牛血清的RPMI中培养3-4天，并用10 µg/mL ConA刺激。收集细胞培养上液，测定大鼠VEGF，测得浓度为1105 pg/mL。

G. 特异性

该检测可识别天然和重组大鼠VEGF。

以下列出的蛋白，用合适的标准品稀释液RD5-18/RD5-3制备成50 ng/mL浓度，并进行交叉反应性检测。在中值大鼠VEGF对照品中，将下列蛋白制备成50 ng/mL，检测是否存在干扰。结果未观察到明显的交叉反应或干扰。

Recombinant rat:		**Recombinant mouse:**
CINC-2α	IL-6	PIGF-2
CINC-2β	IL-10	$VEGF_{115}$
CINC-3	IL-18	$VEGF\text{-}B_{167}$
CNTF	LIX	$VEGF\text{-}B_{186}$
CNTF Rα	MAC/Fc Chimera	VEGF-D
E-Selectin/Fc Chimera	MIP-3α	VEGF R2
EphA5/Fc Chimera	β-NGF	VEGF R3
EphB1/Fc Chimera	Npn-1/Fc Chimera	**Recombinant human:**
Fractalkine	Npn-2/Fc Chimera	$VEGF_{165}$
GDNF	PDGF-AA	$VEGF\text{-}B_{167}$
GM-CSF	PDGF-AB	VEGF-C
IFN-γ	PDGF-BB	VEGF-D

IL-1α	TIMP-1	VEGF R3
IL-1β	TNF-α	**Other recombinants:**
IL-1ra		porcine PDGF
IL-2		zebrafish VEGF
IL-4		

下列蛋白观察到存在交叉反应：

重组因子	**被检测浓度（pg/mL)**	**%交叉率**
Mouse $VEGF_{120}$ (*E. coli*-expressed)	2000	59
Mouse $VEGF_{164}$ (*Sf* 21-expressed)	500	73
Canine $VEGF_{164}$ (*E. coli*-expressed)	2000	14
Human $VEGF_{121}$ (*Sf* 21-expressed)	2000	26
Human $VEGF_{121}$ (*E. coli*-expressed)	2000	31
Human $VEGF_{162}$ (NS0-expressed)	2000	24
Human $VEGF_{206}$ (*E. coli*-expressed)	50,000	0.2
Human VEGF/PIGF	10,000	5.2

下列蛋白观察到存在干扰：

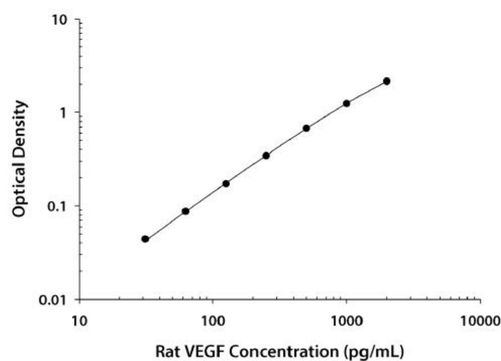
Recombinant mouse VEGF R1	浓度大于200 pg/mL时
Recombinant human VEGF R1	浓度大于1500 pg/mL时
Recombinant human VEGF R2	浓度大于25,000 pg/mL时

IV. 实验

标准曲线实例

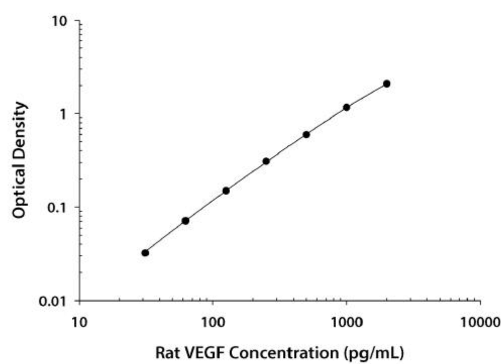
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.118	0.119	—
	0.119		
31.3	0.162	0.163	0.044
	0.164		
62.5	0.201	0.206	0.087
	0.210		
125	0.287	0.290	0.171
	0.293		
250	0.462	0.463	0.344
	0.464		
500	0.791	0.793	0.674
	0.795		
1000	1.313	1.358	1.239
	1.403		
2000	2.237	2.267	2.148
	2.296		

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.112	0.112	—
	0.112		
31.3	0.140	0.144	0.032
	0.147		
62.5	0.179	0.183	0.071
	0.187		
125	0.260	0.261	0.149
	0.262		
250	0.415	0.421	0.309
	0.426		
500	0.698	0.707	0.595
	0.716		
1000	1.240	1.271	1.159
	1.301		
2000	2.128	2.197	2.085
	2.266		

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Rat VEGF Microplate	包被抗大鼠VEGF抗体的96孔聚苯乙烯板，8孔×12条	1块板
Rat VEGF Conjugate	酶标检测抗大鼠VEGF抗体	1瓶
Rat VEGF Standard	重组大鼠VEGF标准品（冻干），参考瓶身标签进行重溶	2瓶
Rat VEGF Control	大鼠VEGF质控品（冻干），质控品的测定值应在标签上规定的范围内	2瓶
Assay Diluent RD1-41	检测液	1瓶
Calibrator Diluent RD5-18	标准品稀释液，*用于细胞培养上清样品*	1瓶
Calibrator Diluent RD5-3	标准品稀释液，*用于大鼠血清/血浆样品*	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	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°C储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="10">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="7">2-8°C储存，最多30天*</td></tr>
<tr><td>检测液RD1-41</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>质控品</td></tr>
<tr><td>标准品</td></tr>
<tr><td>标准品稀释液 RD5-18</td><td>2-8°C 储存，最多 30 天*</td></tr>
<tr><td>标准品稀释液RD5-3</td><td>2-8°C 储存，最多 30 天*</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
- 用于稀释标准品和样品的管子
- 水平振荡器（0.12”轨道），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。

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供参考。样品稳定性未经评估。

细胞培养上清：颗粒物应通过离心去除；立即检测样本或分装，≤ -20 °C储存备用，避免反复冻融。样本可能需要用标准品稀释液RD5-18稀释。

血清样本：血液样品在室温下凝集2小时，然后在1000 × g下离心20分钟。吸取血清样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用标准品稀释液RD5-3稀释。

血浆样本：使用EDTA或肝素作为抗凝剂收集血浆。然后1000 × g离心20分钟。需在30分钟内收集血浆样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用标准品稀释液RD5-3稀释。

注：*本试剂盒对柠檬酸钠血浆尚未被验证。*

严重溶血或脂血样本可能不适用于本试验。

B. 检测前准备工作

使用前请将所有试剂放置于室温。

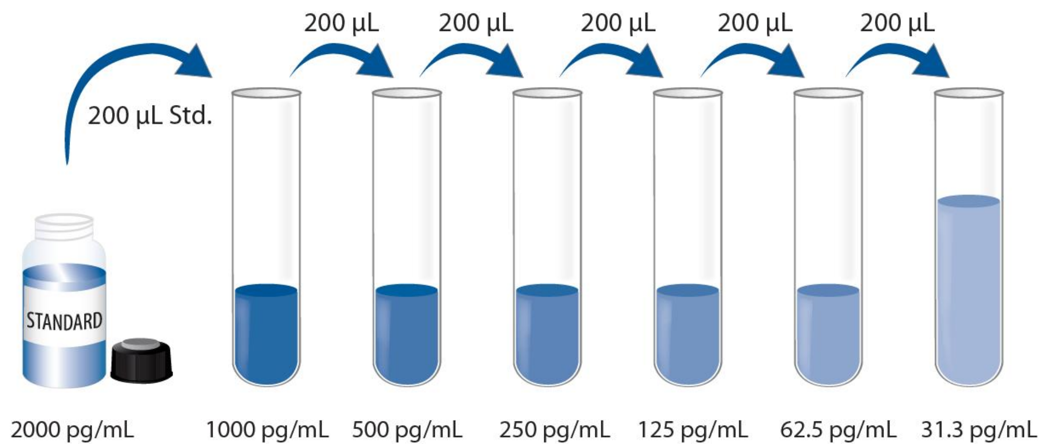
大鼠VEGF质控品：使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，测定时不稀释质控品。

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×）。

大鼠VEGF标准品：复溶体积请参考瓶身标签*。用标准品稀释液RD5-18（*用于细胞培养上清样品*）或标准品稀释液RD5-3（*用于大鼠血清/血浆样品*）复溶大鼠VEGF标准品。请勿使用其他稀释液。此复溶方法得到2000 pg/mL的标准品储备液。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200 µL标准品稀释液RD5-18（*用于细胞培养上清样本*）或标准品稀释液RD5-3（*用于大鼠血清/血浆样本*）吸取到每个管中。使用标准品储备液按下图稀释。在下一次转移之前，将每管彻底混合。未稀释的大鼠VEGF标准品储备液（2000 pg/mL）作为最高标准。标准品稀释液作为零标准（0 pg/mL）。



C. 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、质控品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、标准品、质控品和样本；
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. 每孔加入50 μL检测液RD1-41。
4. 分别将不同浓度标准品、质控品和实验样本加入相应孔中，每孔50 μL。用封板膜封住反应孔，**用水平振荡器（0.12” 轨道）转速：500±50 rpm，室温孵育2小时**。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；
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大鼠VEGF酶标检测抗体。用封板膜封住反应孔，**用水平振荡器（0.12” 轨道）转速：500±50 rpm，室温孵育1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，质控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。数据可以通过绘制大鼠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

1. Conn, G. et al. (1990) J. Biol. Chem. 87:2628.
2. Ishii, H. et al. (2001) Arch. Oral Biol. 46:77.
3. Byrne, A.M. et al. (2005) J. Cell. Mol. Med. 9:777.
4. Robinson, C.J. and S.E. Stringer (2001) J. Cell. Sci. 114:853.
5. Richardson, R.S. et al. (1999) Am. J. Physiol. 277:H2247.
6. Sugishita, Y. et al. (2000) Biochem. Biophys. Res. Commun. 268:657.
7. Yamane, A. et al. (1994) Oncogene 9:2683.
8. Goad, D.L. et al. (1996) Endocrinology 137:2262.
9. Gaudry, M. et al. (1997) Blood 90:4153.
10. Mclaren, J. et al. (1996) J. Clin. Invest. 98:482.
11. Diaz, B.V. et al. (2000) J. Biol. Chem. 275:642.
12. Asano, A. et al. (1997) Biochem. J. 328:179.
13. Bautz, F. et al. (2000) Exp. Hematol. 28:700.
14. Namiki, A. et al. (1995) J. Biol. Chem. 270:31189.
15. Nauck, M. et al. (1997) Am. J. Respir. Cell. Mol. Biol. 16:398.
16. Neufeld, G. et al. (1999) FASEB. J. 13:9.
17. Kowalewski, M.P. et al. (2005) Accession #ABB82619.
18. Pan, Q. et al. (2007) J. Biol. Chem. 282:24049.
19. Weis, S.M. and D.A. Cheresh (2005) Nature 437:497.
20. Breier, G. (2000) Semin. Thromb. Hemost. 26:553.
21. Barleon, B. et al. (1996) Blood 87:3336.
22. Weis, S.M. and D.A. Cheresh (2005) Nature 437:497.
23. Thurston, G. (2002) J. Anat. 200:575.
24. Grothey, A. and E. Galanis (2009) Nat. Rev. Clin. Oncol. 6:507.
25. Carvalho, J.F. et al. (2007) J. Clin. Immunol. 27:246.

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