Quantikine® ELISA

Canine VEGF Immunoassay

Catalog Number CAVE00

For the quantitative determination of canine Vascular Endothelial Growth Factor (VEGF) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

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INTRODUCTION

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). Alternatively 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 exists in dog (5). Isoforms other than $VEGF_{120}$ and $VEGF_{121}$ contain basic heparin-binding regions and are not freely diffusible (4). Canine $VEGF_{164}$ shares 91%, 90%, and 98% aa sequence identity with the rat, mouse, and feline homologs, respectively. VEGF is expressed in multiple cells and tissues including skeletal and cardiac muscle (6, 7), hepatocytes (8), osteoblasts (9), neutrophils (10), macrophages (11), keratinocytes (12), brown adipose tissue (13), $CD34^+$ stem cells (14), endothelial cells (15), fibroblasts, and vascular smooth muscle cells (16). VEGF expression is induced by hypoxia and cytokines such as IL-1, IL-6, IL-8, Oncostatin M and TNF-α (3, 4, 10). 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, 8, 18, 19). 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 (20).

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 (21-23). 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 (24, 25). Various strategies have been employed therapeutically to antagonize VEGF-mediated tumor angiogenesis (26). Circulating VEGF levels correlate with disease activity in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus (27).

The Quantikine Canine VEGF Immunoassay is a 4.5 hour solid phase ELISA designed to measure VEGF levels in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant VEGF and has been shown to accurately quantitate the recombinant factor. Results obtained for naturally occurring canine VEGF showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural canine VEGF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for VEGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any VEGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for 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 VEGF bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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 the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

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.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
VEGF Microplate	890218	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against VEGF.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
VEGF Standard	890220	2 vials of recombinant VEGF in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a fresh standard for each assay. Discard after 4 hours.
VEGF Conjugate	890219	21 mL of a polyclonal antibody against VEGF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5K	895119	21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6U	895148	21 mL of animal serum with preservatives. *For serum/plasma samples. May contain a precipitate. Mix well before and during use.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

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.

PRECAUTIONS

Calibrator Diluent RD6U contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

VEGF is detectable in saliva. Take precautionary measures to prevent contamination of the kit reagents while running the assay.

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.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & 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. Assay immediately or aliquot and store samples 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 1000 x 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 1000 x 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.*

SAMPLE PREPARATION

Use polypropylene tubes.

Plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 150 µL of sample + 150 µL of Calibrator Diluent RD6U.

REAGENT PREPARATION

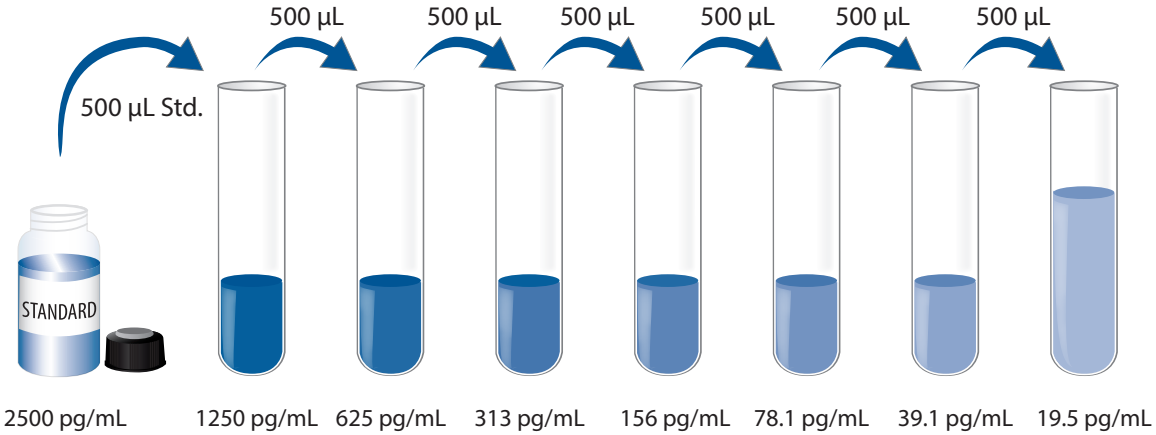
Bring all reagents to room temperature before use.

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

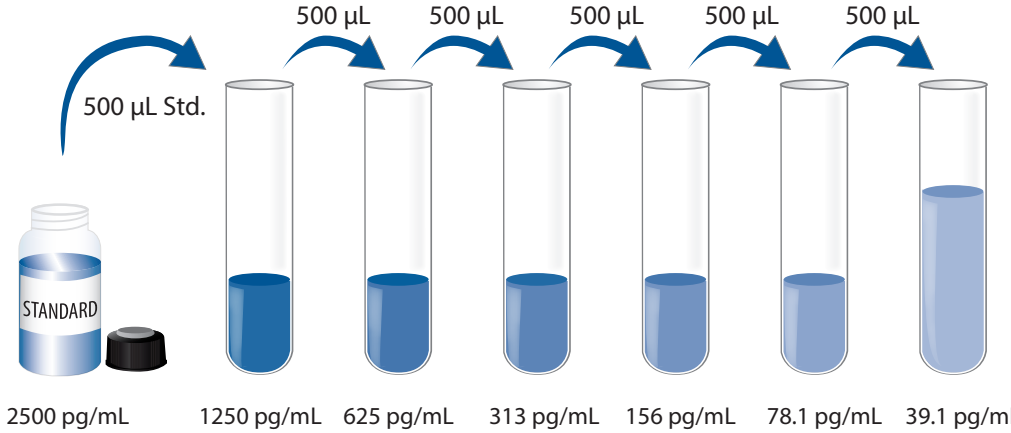
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

VEGF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the VEGF Standard with Calibrator Diluent RD5K (*for cell culture supernate samples*) or Calibrator Diluent RD6U (*for serum/plasma samples*). This reconstitution produces a stock solution of 2500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

For Cell Culture Supernate Samples: Use polypropylene tubes. Pipette 500 µL of Calibrator Diluent RD5K into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1250 pg/mL dilution serves as the high standard. Calibrator Diluent RD5K serves as the zero standard (0 pg/mL).



For Serum/Plasma Samples: Use polypropylene tubes. Pipette 500 µL of Calibrator Diluent RD6U into each tube. *Calibrator Diluent RD6U may contain a precipitate. Mix well before and during use.* Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard stock serves as the high standard (2500 pg/mL). Calibrator Diluent RD6U serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards and samples 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. **For Culture Supernate Samples:** Add 50 µL of Assay Diluent RD1W to each well.
   For Serum/Plasma Samples: Add 100 µL of Assay Diluent RD1W to each well.
4. **For Culture Supernate Samples:** Add 200 µL of Standard, control, or sample per well.
   For Serum/Plasma* Samples: Add 100 µL of Standard, control, or sample* per well.
5. Cover with the adhesive strip provided and incubate for 2 hours at room temperature. A plate layout is provided to record the standards and samples assayed.
6. Aspirate each well and wash, repeating the process twice for a total of three 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.
7. Add 200 µL of VEGF Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
8. Repeat the aspiration/wash as in step 6.
9. Add 200 µL of Substrate Solution to each well. Protect from light.
   For Culture Supernate Samples: Incubate for 20 minutes at room temperature.
   For Serum/Plasma Samples: Incubate for 25 minutes at room temperature.
10. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. 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.

*Plasma samples require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

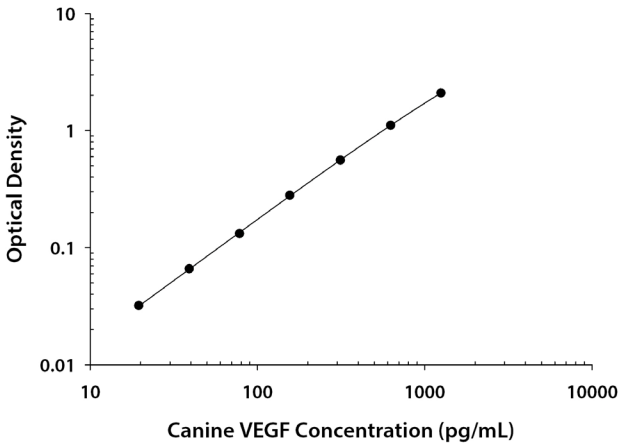
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the canine 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.

TYPICAL DATA

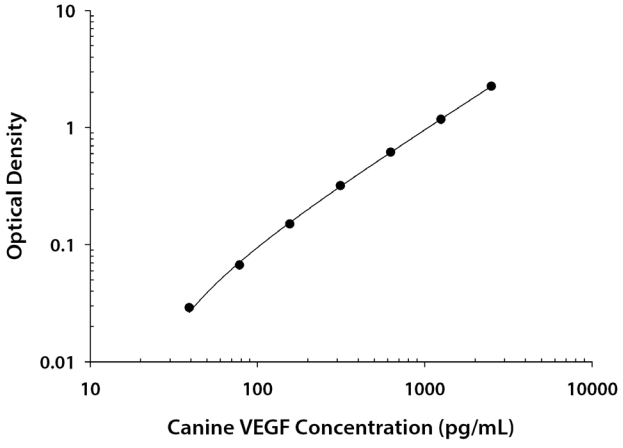
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.009 0.010	0.010	—
19.5	0.040 0.044	0.042	0.032
39.1	0.073 0.078	0.076	0.066
78.1	0.142 0.142	0.142	0.132
156	0.283 0.297	0.290	0.280
313	0.565 0.573	0.569	0.559
625	1.100 1.136	1.118	1.108
1250	2.040 2.161	2.101	2.091

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.010 0.013	0.012	—
39.1	0.040 0.042	0.041	0.029
78.1	0.078 0.080	0.079	0.067
156	0.160 0.163	0.162	0.150
313	0.325 0.337	0.331	0.319
625	0.613 0.644	0.629	0.617
1250	1.131 1.241	1.186	1.174
2500	2.208 2.320	2.264	2.252

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration 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 forty 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	40	40	40
Mean (pg/mL)	29.1	123	531	32.8	128	495
Standard deviation	1.9	5.0	18.4	2.8	6.4	33.0
CV (%)	6.5	4.1	3.5	8.5	5.0	6.7

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	53.7	235	910	64.5	250	1003
Standard deviation	3.6	10.6	46.2	5.7	17.4	61.7
CV (%)	6.7	4.5	5.1	8.8	7.0	6.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	94-110%
Serum (n=4)	103	87-113%
EDTA plasma* (n=4)	98	92-107%
Heparin plasma* (n=4)	107	96-118%

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

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of canine VEGF were diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	104	100	98	97
	Range (%)	100-107	96-107	92-103	88-104
1:4	Average % of Expected	103	100	100	92
	Range (%)	99-106	85-110	94-103	87-96
1:8	Average % of Expected	98	104	98	89
	Range (%)	95-102	103-107	92-105	80-97
1:16	Average % of Expected	99	105	94	93
	Range (%)	97-100	101-111	87-102	81-107

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

SENSITIVITY

Using Calibrator Diluent RD5K the minimum detectable dose (MDD) of canine VEGF is typically less than 9.8 pg/mL. Using Calibrator Diluent RD6U the MDD is typically less than 19.5 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.

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant $VEGF_{165}$ produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Twenty serum, EDTA plasma, and heparin plasma samples were tested for detectable levels of canine VEGF. Four serum samples had measurable levels of canine VEGF; 70.5, 49.1, 55.7, and 70.5 pg/mL. The rest of the samples read below the lowest standard, 39.1 pg/mL. EDTA plasma and heparin plasma samples were not detectable.

Cell Culture Supernates:
Canine peripheral blood leukocytes (PBLs) were cultured in RPMI supplemented with 10% fetal bovine serum, 10 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were stimulated with 10 µg/mL Concanavalin A for 4 days. An aliquot of the cell culture supernate was removed, assayed for canine VEGF, and measured 156 pg/mL.

Canine peripheral blood mononuclear cells (PBMCs) were cultured in RPMI supplemented with 10% fetal bovine serum, 1% HEPES, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were stimulated with 10 µg/mL PHA and 10 ng/mL PMA for 2 days. An aliquot of the cell culture supernate was removed, assayed for canine VEGF, and measured 667 pg/mL.

MDCK canine kidney epithelial cells were cultured in RPMI supplemented with 10% fetal calf serum. The cells were stimulated with 10 µg/mL PHA and 10 ng/mL PMA for 3 days. An aliquot of the cell culture supernate was removed, assayed for canine VEGF, and measured 6996 pg/mL.

Canine lymphoid cells (CLL-1390) were cultured for three days in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1% NEAA (non-essential amino acids), 1% sodium pyruvate, and 0.5% HEPES. An aliquot of the cell culture supernate was assayed and measured 2165 pg/mL.

A-72 canine fibroma cells were cultured for three days in Leibovitz-15 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for canine VEGF, and measured 41.5 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant canine VEGF. This assay also recognizes recombinant human $VEGF_{165}$.

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

Recombinant canine:

CD4
CD8
Epo
GM-CSF
HGF
HGF R
IFN-γ
IL-1β
IL-2
IL-4
IL-5
IL-6
IL-8
IL-10
IL-12
IL-12/IL-23 p40
IL-18
KGF
MCP-1
SCF
TNF-α
TNF RI

Recombinant human:

Neuropilin-1
Neuropilin-2
PlGF
$VEGF_{165}$/PlGF
$VEGF\text{-}B_{167}$
VEGF-C
VEGF-D
VEGF R3

Recombinant mouse:

PlGF-2
$VEGF_{120}$
$VEGF_{164}$
VEGF R2
VEGF R3

Recombinant rat:

Neuropilin-1
Neuropilin-2
VEGF

Recombinant zebrafish:

VEGF

Recombinant human VEGF R1 intereferes at concentrations ≥ 31.3 pg/mL in this assay.

Recombinant human VEGF R2 intereferes at concentrations ≥ 8000 pg/mL in this assay.

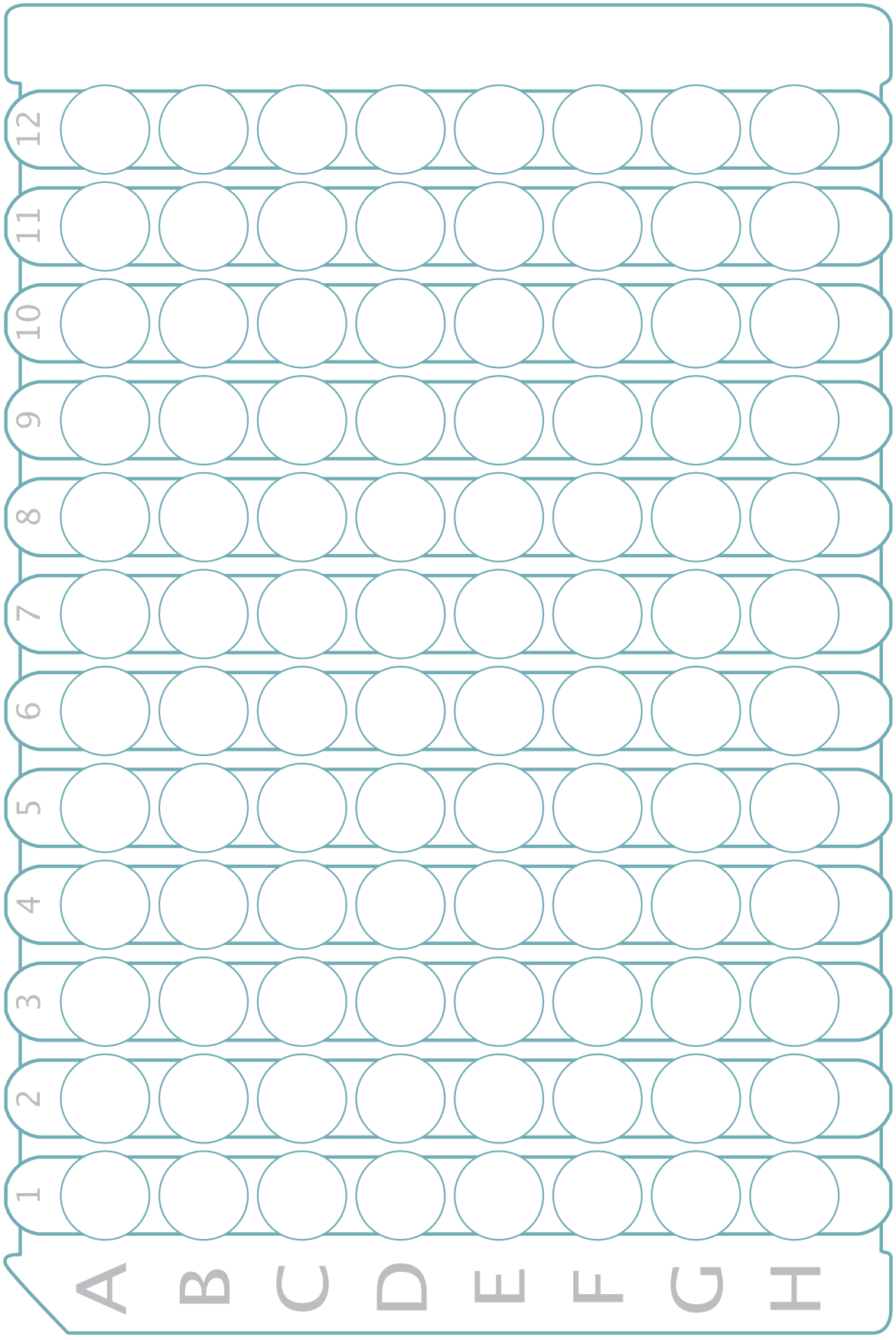
Recombinant mouse VEGF R1 intereferes at concentrations ≥ 125 pg/mL in this assay.

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 Stringer, S.E. (2001) J. Cell. Sci. **114**:853.
5. Scheidegger, P. *et al.* (1999) Biol. Chem. **380**:1449.
6. Richardson, R.S. *et al.* (1999) Am. J. Physiol. **277**:H2247.
7. Sugishita, Y. *et al.* (2000) Biochem. Biophys. Res. Commun. **268**:657.
8. Yamane, A. *et al.* (1994) Oncogene **9**:2683.
9. Goad, D.L. *et al.* (1996) Endocrinology **137**:2262.
10. Gaudry, M. *et al.* (1997) Blood **90**:4153.
11. Mclaren, J. *et al.* (1996) J. Clin. Invest. **98**:482.
12. Diaz, B.V. *et al.* (2000) J. Biol. Chem. **275**:642.
13. Asano, A. *et al.* (1997) Biochem. J. **328**:179.
14. Bautz, F. *et al.* (2000) Exp. Hematol. **28**:700.
15. Namiki, A. *et al.* (1995) J. Biol. Chem. **270**:31189.
16. Nauck, M. *et al.* (1997) Am. J. Respir. Cell. Mol. Biol. **16**:398.
17. Angelo, L.S. and R. Kurzrock (2007) Clin. Cancer Res. **13**:2825.
18. Neufeld, G. *et al.* (1999) FASEB J. **13**:9.
19. Kowalewski, M.P. *et al.* (2005) Accession #ABB82619.
20. Pan, Q. *et al.* (2007) J. Biol. Chem. **282**:24049.
21. Weis, S.M. and D.A. Cheresh (2005) Nature **437**:497.
22. Breier, G. (2000) Semin. Thromb. Hemost. **26**:553.
23. Barleon, B. *et al.* (1996) Blood **87**:3336.
24. Weis, S.M. and D.A. Cheresh (2005) Nature **437**:497.
25. Thurston, G. (2002) J. Anat. **200**:575.
26. Grothey, A. and E. Galanis (2009) Nat. Rev. Clin. Oncol. **6**:507.
27. Carvalho, J.F. *et al.* (2007) J. Clin. Immunol. **27**:246.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES