Quantikine® ELISA

Human VEGF R2/KDR Immunoassay

Catalog Number DVR200
SVR200
PDVR200

For the quantitative determination of human soluble VEGF Receptor 2 (VEGF R2) concentrations in cell culture supernates, cell lysates, 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.

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INTRODUCTION

VEGF R2 [also known as KDR (kinase insert domain receptor) in humans or Flk-1(fetal liver kinase-1) in mice], is a member of the class III subfamily of receptor tyrosine kinases (RTKs) that also includes VEGF R1 (Flt-1) and VEGF R3 (Flt-4). All three receptors contain seven Ig-like repeats within their extracellular domains and kinase insert domains in their intracellular regions. The expression patterns of VEGF R1, VEGF R2, and VEGF R3 are almost exclusively restricted to endothelial cells. These receptors play essential roles in angiogenesis. VEGF R2 binds VEGF-A ($VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$ splice variants), VEGF-C and VEGF-D.

The full-length cDNA for VEGF R2 encodes a 1356 amino acid (aa) precursor protein with a 19 aa signal peptide (1). The mature protein is composed of a 745 aa extracellular domain, a 25 aa transmembrane domain and a 567 aa cytoplasmic domain. The gene for VEGF R2 maps to human chromosome 4q31.2—q32, a locus distinct from locations for other type III growth factor RTKs (2, 3).

VEGF R1, VEGF R2, and VEGF R3 are preferentially expressed in the proliferating endothelium of vessels lining and/or penetrating solid tumors (4). VEGF R2, however, is more widely distributed and expressed in all vessel-derived endothelial cells in comparison to VEGF R1 (5). VEGF R2 is also localized in endothelial cells and perivascular cells of capillaries within the lamina propria of seminiferous tubules, Leydig cells and Sertoli cells (6).

In contrast to VEGF R1, which binds both PlGF and VEGF with high affinity, VEGF R2 binds VEGF but not PlGF with high affinity (7). *In vitro* studies further demonstrate that PlGF/VEGF heterodimers can bind with high affinity to soluble VEGF R2, but PlGF homodimers fail to bind this receptor (8). Soluble forms of VEGF R1 and VEGF R2 also differ significantly from one another in terms of their abilities to block VEGF-induced cell proliferation and migration (9). Soluble VEGF R2 cannot compete with VEGF for binding to human endothelial cells expressing both VEGF R1 and VEGF R2, in contrast to soluble VEGF R1. Soluble VEGF R2 can only partially inhibit cell migration, whereas soluble VEGF R1 can almost completely block VEGF-induced cell proliferation and migration (9). The binding of VEGF to soluble VEGF R2, but not VEGF R1, is also dependent on heparin (9).

The VEGF/VEGF R2 signaling pathway plays an important role in tumor angiogenesis and other diseases where "pathological angiogenesis" is involved. Inactivation of functional VEGF R2 by a blocking antibody can disrupt angiogenesis and prevent tumor cell invasion (10,11). Angiogenesis induced by the HIV-1 Tat protein is mediated by VEGF R2 on vascular endothelial cells (12). Tat specifically binds and activates VEGF R2. Tat-induced angiogenesis is blocked by agents that can block VEGF R2 (12,13).

The Quantikine Human VEGF R2/KDR Immunoassay is a 4.5 hour solid-phase Immunoassay designed to measure human soluble VEGF R2 in cell culture supernates, cell lysates, serum, and plasma. It contains NS0-expressed recombinant human VEGF R2/Fc Chimera and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human VEGF R2 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 naturally occurring human soluble VEGF R2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for VEGF R2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any VEGF R2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for VEGF R2 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 R2 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 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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 #	CATALOG # DVR200	CATALOG # SVR200	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
VEGF R2 Microplate	890930	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against VEGF R2.	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.*
VEGF R2 Conjugate	890931	1 vial	6 vials	21 mL/vial of polyclonal antibody against VEGF R2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
VEGF R2 Standard	890932	1 vials	6 vials	50 ng/vial of recombinant human VEGF R2 in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1W	895117	1 vial	6 vials	11 mL/vial of a buffered protein solution with preservatives.	
Cell Lysis Buffer 2	895347	2 vials	12 vials	21 mL/vial of a buffered solution with preservatives.	
Calibrator Diluent RD6-31	895323	2 vials	12 vials	21 mL/vial of a buffered animal serum with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DVR200 contains sufficient materials to run an ELISA on one 96 well plate.
SVR200 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDVR200). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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.
- Centrifuge
- Phosphate-buffered saline (for cell lysis procedure).
- Test tubes for dilution of standards and samples.
- Human VEGF R2 Controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® 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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 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

Serum and plasma samples require a 5-fold dilution. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD6-31.

Cells from culture extracts must be lysed before assaying according to the following directions.

1. Wash cells three times in cold PBS.
2. Resuspend cells in Cell Lysis Buffer 2 to a concentration of 1.5 x 10^6 cells/mL.
3. Incubate for 1 hour at room temperature with gentle mixing.
4. Centrifuge cells at 1000 x g for 15 minutes.
5. Assay the supernate immediately or aliquot and store at ≤ -70 °C.

REAGENT PREPARATION

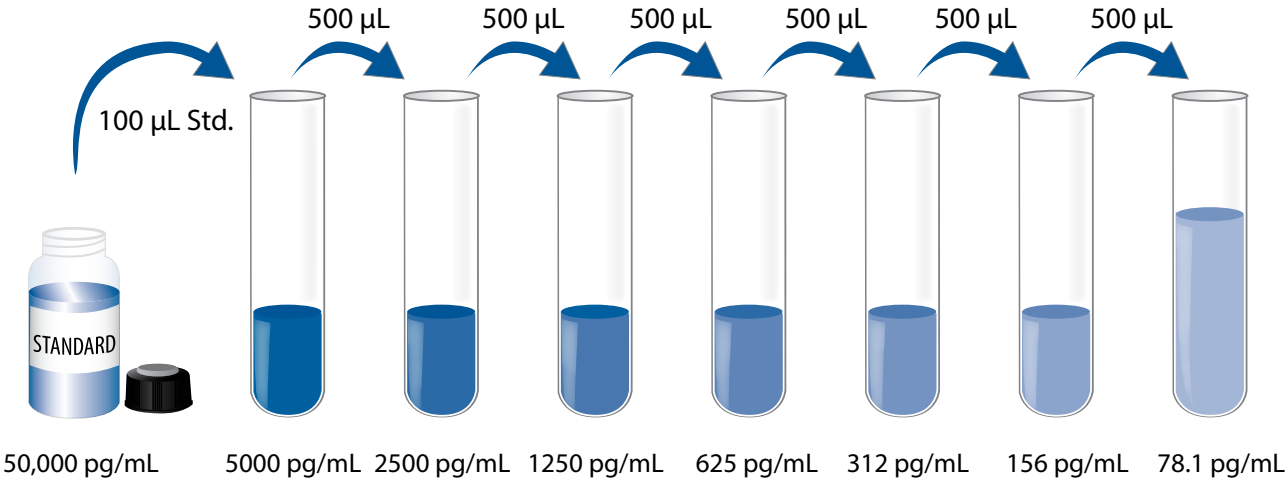
Bring all reagents to room temperature before use.

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 R2 Standard - Reconstitute the VEGF R2 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 50,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD6-31 into the 5000 pg/mL tube. Pipette 500 µL of Calibrator Diluent RD6-31 into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. The Calibrator Diluent 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, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1W to each well.
4. Add 100 µL of Standard, controls, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of VEGF R2 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 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 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 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.

*Samples may require dilution and/or lysis. See Sample Preparation.

CALCULATION OF RESULTS

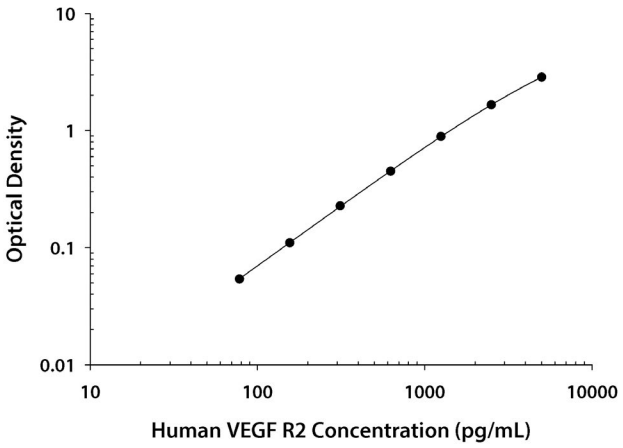
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 VEGF R2 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

This 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	0.012 0.012	0.012	—
78.1	0.066 0.066	0.066	0.054
156	0.124 0.121	0.122	0.110
312	0.241 0.237	0.239	0.227
625	0.471 0.454	0.462	0.450
1250	0.906 0.895	0.900	0.888
2500	1.676 1.664	1.670	1.658
5000	2.898 2.843	2.870	2.858

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	465	1269	2995	455	1233	2962
Standard deviation	19.4	45.5	87.9	32.1	84.8	169
CV (%)	4.2	3.6	2.9	7.0	6.9	5.7

RECOVERY

The recovery of VEGF R2 spiked to levels throughout the range of the assay in cell culture media was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	99	92-104%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of VEGF R2 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were treated as directed in the Sample Preparation section.

		Cell lysates (n=2)	Serum (n=6)	EDTA plasma (n=6)	Heparin plasma (n=6)
1:2	Average % of Expected	108	101	101	104
	Range (%)	107-109	100-102	98-105	102-106
1:4	Average % of Expected	110	102	99	106
	Range (%)	109-111	100-104	95-104	102-110
1:8	Average % of Expected	104	105	102	105
	Range (%)	103-105	102-106	96-109	102-109
1:16	Average % of Expected	———	103	98	101
	Range (%)	———	97-111	93-102	100-102

SENSITIVITY

Fifty-one assays were evaluated and the minimum detectable dose (MDD) of VEGF R2 ranged from 1.0-11.4 pg/mL. The mean MDD was 4.6 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 NS0-expressed recombinant human VEGF R2/Fc Chimera produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma -Samples from apparently healthy volunteers were diluted 5-fold in Calibrator Diluent RD6-31 and evaluated for the presence of VEGF R2 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=60)	9768	6420-14,501	1803
EDTA Plasma (n=35)	9577	6635-13,553	1616
Heparin Plasma (n=35)	9584	6400-12,354	1387

Cell Culture Supernates/Lysates - HUVEC human umbilical vein endothelial cells (5×10^6 cells/mL) were cultured in EGM supplemented with 2% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and bovine brain extract. Cells were grown to confluence, trypsinized, and the supernate was poured off and assayed. Cells were resuspended in Cell Lysis Buffer 2 at 1.5×10^6 cells/mL and the lysate was assayed.

Condition	(pg/mL)
HUVEC Lysate	990
HUVEC Supernate	210

SPECIFICITY

This assay recognizes natural and recombinant human VEGF R2.

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 recombinant human VEGF R2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

β-ECGF
EGF
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
FGF-9
FGF-10
FGF-18
Flt-3
Flt-3/Flk-2 Ligand
G-CSF
GM-CSF
HB-EGF
HGF
HRG-α
IGF-I
IGF-II
KGF (FGF-7)
M-CSF
MSP
MSP β
β-NGF
PDGF-AA
PDGF-AB
PDGF-BB
PD-ECGF
PlGF
$VEGF_{121}$
$VEGF_{165}$
VEGF/PlGF
VEGF-D
VEGF R1 (Flt-1)
VEGF R3 (Flt-4)

Recombinant mouse:

FGF-8b
FGF-8c
Flt-3/Flk-2 Ligand
G-CSF
GM-CSF
M-CSF
PlGF-2
VEGF R1 (Flt-1)
VEGF R2 (Flk-1)

Recombinant rat:

GM-CSF
β-NGF
PDGF-BB

Recombinant porcine:

GM-CSF

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF

Recombinant mouse $VEGF_{120}$ interferes at concentrations > 25 ng/mL in a serum sample.

Recombinant mouse $VEGF_{164}$ interferes at concentrations > 10 ng/mL in a serum sample.

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