Human sVEGF-R1 Platinum ELISA

Catalog #: ALX-850-264

Enzyme-linked Immunosorbent Assay for quantitative detection of human sVEGF-R1.

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

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INTENDED USE

The human sVEGF-R1 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human sVEGF-R1. **The human SVEGF-R1 ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

SUMMARY

Soluble VEGF-R1 (sFLT-1) is a naturally occurring endogenous form of the VEGF-R1 and was originally found in the supernatant of human vascular endothelial cells. It is generated by differential splicing of the *flt-1* gene. In vitro sVEGF-R1 is used to inhibit VEGF-A mediated signals in endothelial cells and in vivo it can be used to block physiological angiogenesis in several organs, e.g. in the ovary or in bones. Tumor cells transfected with the *flt-1* gene are growth restricted in vivo because of the limitation in developing tumor blood vessels via VEGF-A signaling. Very recent studies have shown that this molecule is present endogenously atng/ml concentrations in biologicals fluids of normal human subjects or in the conditioned media of FLT-1 positive cell types. The measurement of sFLT-1 in a variety of clinical conditions may open up new insights in health and disease.

Characterization of angiogenic activity, such as embryonic development, placental vascularization, cancer and wound healing is measured by comparing the ratio of angiogenic stimulators (e.g. FGF-2, FGF-1, VEGF-A, Ang-1) to angiogenic inhibitors (e.g. sFLT-1, angiostatin, endostatin, thrombospondin). Several independently published data of both normal and pathogenic subjects have confirmed endogenous levels of VEGF-A and bFGF in pg/ml ranges. These factors have been thought to work unopposed to cause blood vessel formation. The finding that sVEGF-R1, a strong VEGF-A antagonist, is present in normal subjects suggests a finely tuned balance of signal transduction, the workings of which can now be explored. Together with other similar assay systems, positive and negative angiogenic regulators can now be explored in many different physiological and pathological settings using human cell culture supernatants and biologicals fluids.

For literature update refer to **www.enzolifesciences.com**

PRINCIPLES OF THE TEST

|	
An anti-human sVEGF-R1 coating antibody is adsorbed onto microwells.	**Coated Microwell** Coating Antibody
Human sVEGF-R1 present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human sVEGF-R1 antibody is added and binds to human sVEGF-R1 captured by the first antibody.	**First Incubation** Standard or Sample Biotin-Conjugate
Following incubation unbound biotin-conjugated anti-human sVEGF-R1 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human sVEGF-R1 antibody.	**Second Incubation** Streptavidin-HRP
Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.	**Third Incubation** Substrate
A colored product is formed in proportion to the amount of human sVEGF-R1 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from 7 human sVEGF-R1 standard dilutions and human sVEGF-R1 sample concentration determined.	Reacted Substrate
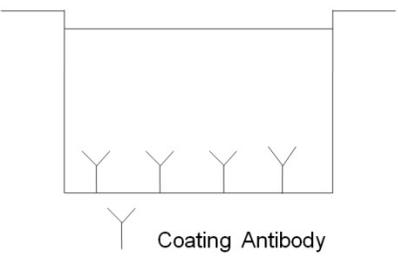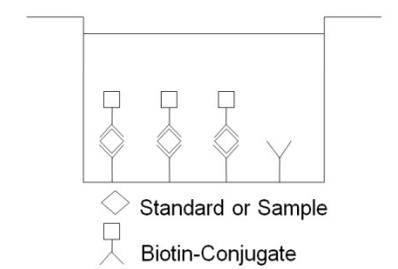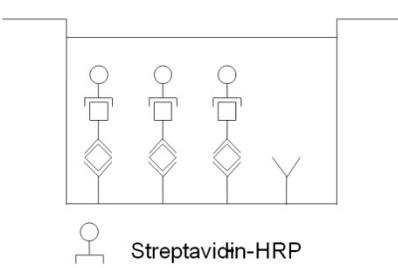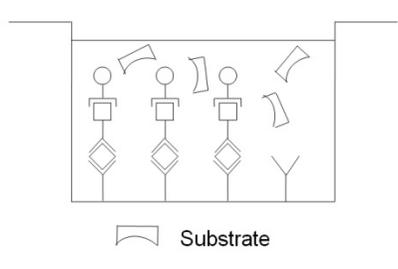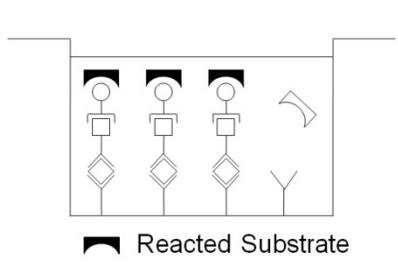
REAGENTS PROVIDED

96 tests

- 1 foil pouch with a Microwell Plate coated with monoclonal antibody to human sVEGF-R1

- 1 vial (70µl) Biotin-Conjugate anti-human sVEGF-R1 polyclonal antibody
- 1 vial (150µl) Streptavidin-HRP
- 2 vials human sVEGF-R1 Standard lyophilized, 20ng/ml upon reconstitution
- 1 bottle (20ml) Conjugate Diluent
- 1 vial (5ml) Assay Buffer Concentrate 20x (PBS with 1% Tween 20 and 10% BSA)
- 1 bottle (50ml) Wash Buffer Concentrate 20x (PBS with 1% Tween 20)
- 1 vial (15ml) Substrate Solution (tetramethyl-benzidine)
- 1 vial (15ml) Stop Solution (1M Phosphoric acid)
- 4 Adhesive Films

Storage Instructions–ELISA Kit

Store kit reagents between 2-8°C. Immediately after use remaining reagents should be returned to cold storage (2-8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Specimen Collection and Storage Instructions

Cell culture supernatant, serum and plasma (EDTA, citrate), were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Pay attention to a possible **"Hook Effect"** due to high sample concentrations (see **Calculation of Results**, page 13).

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human sVEGF-R1. If samples are to be run within 24 hours, they may be stored at 2-8°C (for sample stability refer to **Sample Stability**, page 18).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

OTHER MATERIALS NEEDED

- 5ml and 10ml graduated pipettes
- 5µl to 1000µl adjustable single channel micropipettes with disposable tips
- 50µl to 300µl adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450nm (620nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

PRECAUTIONS FOR USE

Handle with care

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.

- Do not eat or smoke in areas where kit reagents or samples are handled.

- Avoid contact of skin or mucous membranes with kit reagents or specimens.

- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.

- Avoid contact of substrate solution with oxidizing agents and metal.

- Avoid splashing or generation of aerosols.

- In order to avoid microbial contamination or cross-contamination of reagents or specimens, which may invalidate the test, use disposable pipette tips and/or pipettes.

- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.

- Exposure to acid inactivates the conjugate.

- Glass-distilled water or deionized water must be used for reagent preparation.

- Substrate solution must be at room temperature prior to use.

- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.

- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

REAGENT PREPARATION

Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.

If crystals have formed in the **Buffer Concentrates**, warm them gently until they have completely dissolved.

Wash Buffer (1x)

Pour entire contents (50ml) of the **Wash Buffer Concentrate** (20x) into a clean 1000ml graduated cylinder. Bring to final volume of 1000ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2-25°C. Please note that Wash Buffer (1x) is stable for 30 days.

Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1-6	25	475
1-12	50	950

Assay Buffer (1x)

Pour the entire contents (5ml) of the **Assay Buffer Concentrate** (20x) into a clean 100ml graduated cylinder. Bring to final volume of 100ml with distilled water. Mix gently to avoid foaming.

Store at 2-8°C. Please note that the Assay Buffer (1x) is stable for 30 days.

Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1-6	2.5	47.5
1-12	5.0	95.0

Biotin-Conjugate

Please note *that the Biotin-Conjugate should be used within **30 minutes** after dilution.*

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** solution with Conjugate Diluent in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Conjugate Diluent (ml)
1-6	0.03	2.97
1-12	0.06	5.94

Streptavidin-HRP

Please note *that the Streptavidin-HRP should be used **within 30 minutes** after dilution.*

Make a 1:100 dilution of the concentrated **Streptavidin-HRP** solution with Conjugate Diluent in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Conjugate Diluent (ml)
1-6	0.06	5.94
1-12	0.12	11.88

Human sVEGF-R1 Standard

- Reconstitute **human sVEGF-R1 standard** by addition of distilled water.

- Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization

  (concentration of reconstituted standard = 20.00ng/ml).

- Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

- After usage remaining standard cannot be stored and has to be discarded.

External Standard Dilution

− Label 7 tubes, one for each standard point.

S1, S2, S3, S4, S5, S6, S7

− Then prepare 1:2 serial dilutions for the standard curve as follows:

− Pipette 225µl of Assay Buffer (1x) into each tube.

− Pipette 225µl of reconstituted standard (concentration of standard = 20ng/ml) into the first tube, labeled S1, and mix (concentration of standard 1 = 10ng/ml).

− Pipette 225µl of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.

− Repeat serial dilutions 5 more times thus creating the points of the standard curve (see **Figure 1**).

− Assay Buffer (1x) serves as blank.

Figure 1

Transfer 225 µl

S1   S2   S3   S4   -   S7

Reconstituted
sVEGF-R Standard

Assay Buffer (1x)
225 µl

Discard
225 µl
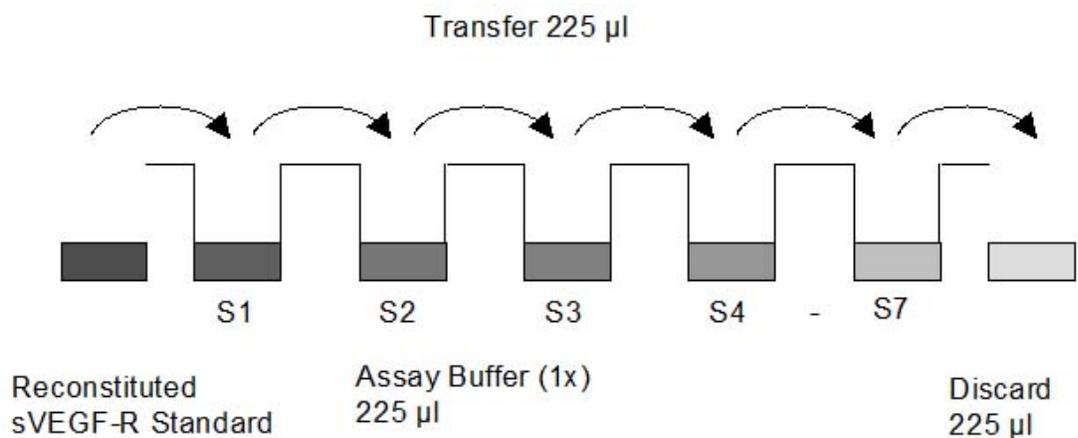
TEST PROTOCOL

a. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8°C sealed tightly.

b. Wash the microwell strips twice with approximately 400µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10–15 seconds** before aspiration. Take care not to scratch the surface of the microwells. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. **Do not allow wells to dry**.

c. <u>**Standard dilution on the microwell plate**</u> (Alternatively the standard dilution can be prepared in tubes: see **External Standard Dilution**, page 9.):

Add 100µl of Assay Buffer (1x) in duplicate to all **standard wells**. Pipette 100µl of prepared **standard** (see **Preparation of Standard**, page 8, concentration = 20.00ng/ml) in duplicate into well A1 and A2 (see **Table 1**). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 10.00ng/ml), and transfer 100µl to wells B1 and B2, respectively (see **Figure 2**). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human SVEGF-R1 standard dilutions ranging from 10.00 to 0.16ng/ml. Discard 100µl of the contents from the last microwells (G1, G2) used.

Figure 2
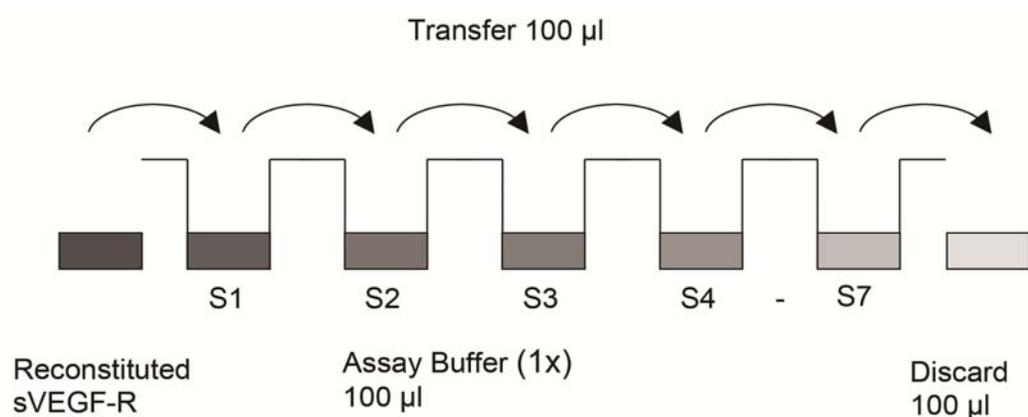
In case of an **external standard dilution** (see page 9), pipette 100µl of these standard dilutions (S1-S7) in the standard wells according to **Table 1**.

Table 1 depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3		3
A	Standard 1 (10.00ng/ml)	Standard 1 (10.00ng/ml)	Sample 1	Sample 1	Sample 1
B	Standard 2 (5.00ng/ml)	Standard 2 (5.00ng/ml)	Sample 2	Sample 2	Sample 2
C	Standard 3 (2.50ng/ml)	Standard 3 (2.50ng/ml)	Sample 3	Sample 3	Sample 3
D	Standard 4 (1.25ng/ml)	Standard 4 (1.25ng/ml)	Sample 4	Sample 4	Sample 4
E	Standard 5 (0.63ng/ml)	Standard 5 (0.63ng/ml)	Sample 5	Sample 5	Sample 5
F	Standard 6 (0.32ng/ml)	Standard 6 (0.32ng/ml)	Sample 6	Sample 6	Sample 6
G	Standard 7 (0.16ng/ml)	Standard 7 (0.16ng/ml)	Sample 7	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8	Sample 8

d. Add 100µl of **Assay Buffer (1x)** in duplicate to the **blank wells**.

e. Add 50µl of **Assay Buffer (1x)** to the **sample wells**.

f. Add 50µl of each **sample** in duplicate to the **sample wells**.

g. Prepare **Biotin-Conjugate** (see **Preparation of Biotin-Conjugate**, page 8).

h. Add 50µl of **Biotin-Conjugate** to all wells.

i. Cover with an adhesive film and incubate at room temperature (18-25°C) for 2 hours, if available on a microplate shaker set at 100 rpm.

j. Prepare **Streptavidin-HRP** (refer to **Preparation of Streptavidin-HRP**, page 8).

k. Remove adhesive film and empty wells. **Wash** microwell strips 6 times according to point b. of the test protocol. Proceed immediately to the next step.

l. Add 100µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.

m. Cover with an adhesive film and incubate at room temperature (18-25°C) for 1 hour, if available on a microplate shaker set at 100rpm.

n. Remove adhesive film and empty wells. **Wash** microwell strips 6 times according to point b. of the test protocol. Proceed immediately to the next step.

o. Pipette 100µl of **TMB Substrate Solution** to all wells.

p. Incubate the microwell strips at room temperature (18-25°C) for about 30 min. Avoid direct exposure to intense light.

   The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable.

   Determination of the ideal time period for color development has to be done individually for each assay.

   It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9–0.95.

q. Stop the enzyme reaction by quickly pipetting 100µl of **Stop Solution** into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2-8°C in the dark.

r. Read absorbance of each microwell on a spectrophotometer using 450nm as the primary wave length (optionally 620nm as the reference wave length; 610nm to 650nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

   Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human sVEGF-R1 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

- To determine the concentration of circulating human sVEGF-R1 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human sVEGF-R1 concentration.

- **If instructions in this protocol have been followed samples have been diluted 1:2 (50µl sample + 50µl Assay Buffer (1x)), the concentration read from the standard curve must be multiplied by the dilution factor (x 2).**

- **Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human sVEGF-R1 levels (Hook Effect). Such samples require further external predilution according to expected human sVEGF-R1 values with Assay Buffer (1x) in order to precisely quantitate the actual human sVEGF-R1 level.**

- It is suggested that each testing facility establishes a control sample of known human sVEGF-R1 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

- A representative standard curve is shown in **Figure 3**. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3

Representative standard curve for human sVEGF-R1 ELISA. Human sVEGF-R1 was diluted in serial 2-fold steps in Assay Buffer (1x). Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.
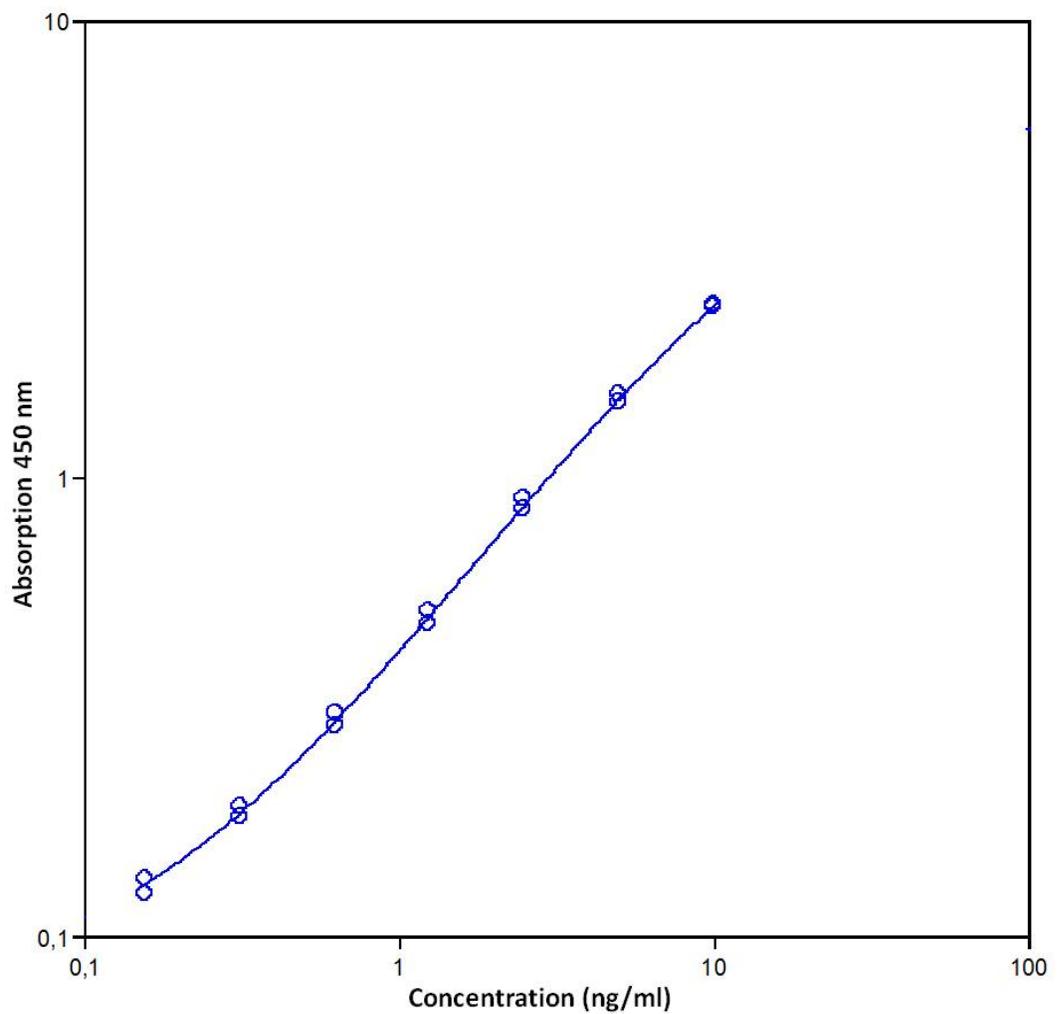
Table 2

Typical data using the human SVEGF-R1 ELISA
Measuring wavelength: 450nm
Reference wavelength: 620nm

Standard	Human sVEGF-R1 Concentration (ng/ml)	O.D. at 450nm	Mean O.D. at 450nm	C.V. (%)
1	10.00	2.389	2.381	0.4
		2.372		
2	5.00	1.458	1.488	2.0
		1.518		
3	2.50	0.900	0.876	2.8
		0.852		
4	1.25	0.511	0.496	3.1
		0.480		
5	0.63	0.306	0.297	3.0
		0.289		
6	0.31	0.191	0.186	2.9
		0.181		
7	0.16	0.134	0.129	3.8
		0.124		
Blank	0.00	0.054	0.054	1.6
		0.055		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

LIMITATIONS

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.

- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.

- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

PERFORMANCE CHARACTERISTICS

Sensitivity

The limit of detection of human sVEGF-R1 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.03ng/ml (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human sVEGF-R1. 2 standard curves were run on each plate. Data below show the mean human sVEGF-R1 concentration and the coefficient of variation for each sample (see **Table 3**). The calculated overall intra-assay coefficient of variation was 5.5%.

Table 3

The mean human sVEGF-R1 concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Human sVEGF-R1 Concentration (ng/ml)	Coefficient of Variation (%)
1	1	15.38	3.5
	2	14.15	5.4
	3	15.26	2.9
2	1	9.91	3.2
	2	9.16	4.6
	3	9.86	3.5
3	1	5.46	6.2
	2	5.23	2.8
	3	5.47	5.3
4	1	4.05	5.2
	2	3.71	3.9
	3	3.95	5.5
5	1	2.87	6.2
	2	2.40	4.6
	3	2.90	7.8
6	1	2.15	5.9
	2	2.04	4.8
	3	2.28	5.4
7	1	0.52	2.6
	2	0.46	8.9
	3	0.50	11.8
8	1	0.82	5.4
	2	0.85	7.0
	3	0.86	8.8

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 2 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human sVEGF-R1. 2 standard curves were run on each plate. Data below show the mean human sVEGF-R1 concentration and the coefficient of variation calculated on 18 determinations of each sample (see **Table 4**).

The calculated overall inter-assay coefficient of variation was 5.1%.

Table 4

The mean human sVEGF-R1 concentration and the coefficient of variation of each sample

Sample	Mean Human sVEGF-R1 Concentration (ng/ml)	Coefficient of Variation (%)
1	14.93	4.5
2	9.64	4.3
3	5.39	2.5
4	3.90	4.5
5	2.72	10.4
6	2.16	5.5
7	0.49	6.5
8	0.84	2.4

Spike Recovery

The spike recovery was evaluated by spiking 3 levels of human sVEGF-R1 into serum, plasma and cell culture supernatant. Recoveries were determined with 4 replicates each. The amount of endogenous human sVEGF-R1 in unspiked samples was subtracted from the spike values.

Recoveries were shown to depend on the serum used.

For recovery data see **Table 5**.

Table 5

Sample matrix	Spike high (%)	Spike medium (%)	Spike low (%)
Serum	46	50	67
Plasma (EDTA)	73	72	91
Plasma (citrate)	148	115	155
Cell culture supernatant	47	56	79

Dilution Parallelism

Serum, plasma and cell culture supernatant samples with different levels of human sVEGF-R1 were analyzed at serial 2 fold dilutions with 4 replicates each.

For recovery data see **Table 6.**

Table 6

Sample matrix	Recovery of Exp. Val.	
	Range (%)	Mean (%)
Serum	112-148	128
Plasma (EDTA)	114-142	125
Plasma (citrate)	77-121	102
Cell culture supernatant	105-127	116

Sample Stability

Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human sVEGF-R1 levels determined. There was no significant loss of human sVEGF-R1 immunoreactivity detected by freezing and thawing.

Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human sVEGF-R1 level determined after 24 h. There was no significant loss of human sVEGF-R1 immunoreactivity detected during storage at -20°C and 4°C.

A significant loss of human sVEGF-R1 immunoreactivity was detected during storage at RT and 37°C.

Specificity

The assay detects both natural and recombinant human sVEGF-R1.

The cross reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human sVEGF-R1 positive sample. Interference was detected for VEGF-A at concentrations >0.15ng/ml and for PLGF-1 at concentrations >2.5ng/ml.

There was no cross reactivity or interference detected for KDR, PDGFAA, PDGF-BB, VEGF-B, VEGF-C, VEGF-D, HGF, EGF.

Expected Values

Panels of 40 serum as well as EDTA and citrate plasma samples from randomly selected apparently healthy donors (males and females) were tested for human sVEGF-R1. The levels measured may vary with the sample collection used.

Elevated human sVEGF-R1 levels depend on the type of immunological disorder.

For detected human sVEGF-R1 levels see **Table 7**.

Table 7

Sample Matrix	Number of Samples Evaluated	Range (ng/ml)	% Detectable	Mean of Detectable (ng/ml)
Serum	40	nd *-0.42	5	--
Plasma (EDTA)	40	nd	--	--
Plasma (Citrate)	40	nd	--	--

* n.d. = non-detectable, samples measured below the lowest standard point are considered to be non-detectable.

REAGENT PREPARATION SUMMARY

Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20x (50ml) to 950ml distilled water.

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1-6	25	475
1-12	50	950

Assay Buffer (1x)

Add **Assay Buffer Concentrate** 20x (5ml) to 95ml distilled water.

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1-6	2.5	47.5
1-12	5.0	95.0

Biotin-Conjugate

Make a 1:100 dilution of **Biotin-Conjugate** in Conjugate Diluent:

Number of Strips	Biotin-Conjugate (ml)	Conjugate Diluent (ml)
1-6	0.03	2.97
1-12	0.06	5.94

Streptavidin-HRP

Make a 1:100 dilution of **Streptavidin-HRP** in Conjugate Diluent:

Number of Strips	Streptavidin-HRP (ml)	Conjugate Diluent (ml)
1-6	0.06	5.94
1-12	0.12	11.88

Human sVEGF-R1 Standard

Reconstitute lyophilized **human sVEGF-R1 standard** with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

TEST PROTOCOL SUMMARY

1. Determine the number of microwell strips required.

2. Wash microwell strips twice with Wash Buffer.

3. Standard dilution on the microwell plate: Add 100µl Assay Buffer (1x), in duplicate, to all standard wells. Pipette 100µl prepared standard into the first wells and create standard dilutions by transferring 100µl from well to well. Discard 100µl from the last wells.

    Alternatively external standard dilution in tubes (see page 9): Pipette 100µl of these standard dilutions in the microwell strips.

4. Add 100µl Assay Buffer (1x), in duplicate, to the blank wells.

5. Add 50µl Assay Buffer (1x) to sample wells.

6. Add 50µl sample in duplicate, to designated sample wells.

7. Prepare Biotin-Conjugate.

8. Add 50µl Biotin-Conjugate to all wells.

9. Cover microwell strips and incubate 2 hours at room temperature (18-25°C).

10. Prepare Streptavidin-HRP.

11. Empty and wash microwell strips 6 times with Wash Buffer.

12. Add 100µl diluted Streptavidin-HRP to all wells.

13. Cover microwell strips and incubate 1 hour at room temperature (18-25°C).

14. Empty and wash microwell strips 6 times with Wash Buffer.

15. Add 100µl of TMB Substrate Solution to all wells.

16. Incubate the microwell strips for about 30 minutes at room temperature (18-25°C).

17. Add 100µl Stop Solution to all wells.

18. Blank microwell reader and measure color intensity at 450nm.

    Note: If instructions in this protocol have been followed samples have been diluted 1:2 (50µl sample + 50µl Assay Buffer (1x)), the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

NOTES

NOTES

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