HUMAN SOLUBLE VEGF-R II ELISA KIT

For the quantitative determination of human sVEGF-R II concentrations in cell culture supernates, serum, and plasma

PURCHASE INFORMATION:

ELISA NAME	HUMAN SOLUBLE VEGF-R II ELISA
Catalog No.	SK00123-02
Lot No.	
Formulation	96 T
Standard Range	31-2000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma, cell culture
Specificity	Human sVEGF-R II
Sample Dilution	20 ~ 40 (*Optimal dilutions should be determined by each laboratory for each application*)
Intra-assay Precision	6-8%
Inter-assay Precision	8-12%
Storage	2 °C-8 °C

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Human soluble VEGF-R II immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human sVEGF-R II in cell culture supernates, serum, and plasma. It contains recombinant human sVEGF-R II and antibodies raised against this protein. It has been shown to accurately quantify recombinant human sVEGF-R II. Results obtained with naturally occurring sVEGF-R II samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the Immunoassay kit can be used to determine relative mass values for natural human sVEGF-R II.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for sVEGF-R II has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sVEGF-R II present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for sVEGF-R II is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of sVEGF-R II bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ 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.

_ It is important that the DILUTION BUFFER selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with dilution buffer 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.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
sVEGF-RII Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against sVEGF-R II.	**123-02-01**	**1 plate**
sVEGF-R II Standard – 1000 pg/vial of recombinant human sVEGF-R II in a buffered protein base with preservatives; lyophilized.	**123-02-02**	**2 vials**
Detection Antibody Concentrate– 105 µL / vial, 100-fold concentrated of Biotinylated polyclonal antibody against sVEGF-R II with preservatives; lyophilized.	**123-02-03**	**1 vial**
Positive Control- one of recombinant human sVEGF-R II, lyophilized	**123-02-04**	**1 vial**
Streptavidin-HRP Conjugate -75 ul/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer- 60mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Antibody Diluent Solution Concentrate – 11mL of buffered protein based solution with preservatives	**DB20**	**1 bottle**
Wash Buffer -50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution-11 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution（0.5M HCl）, 11 mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 6 months. For longer storage, unopened Standard, Positive Control, Detection Antibody Concentrate should be

stored at -20°C or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard and Antibody Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrate and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

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 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, heparin, or citrate 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: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

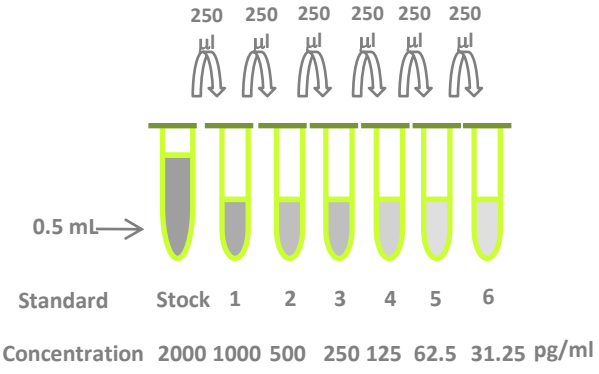
SAMPLE PREPARATION

Serum and plasma samples may require a 20~40 fold dilution. A suggested 20-fold dilution is 15 µL sample + 285 µL Dilution Buffer. A suggested 40-fold dilution is 10 µL sample + 390 µL Dilution Buffer.
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.
sVEGF-R II Standard - Refer to vial label for reconstitution volume. Reconstitute the **sVEGF-R II** Standard with 0.5 ml of Dilution Buffer. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of the appropriate Dilution Buffer into the tube #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	500 µl	2000 pg/ml
# 1	250 µl of stock	250 µl	1000 pg/ml
# 2	250 µl of 1	250 µl	500 pg/ml
# 3	250 µl of 2	250 µl	250 pg/ml
# 4	250 µl of 3	250 µl	125 pg/ml
# 5	250 µl of 4	250 µl	62.5 pg/ml
# 6	250 µl of 5	250 µl	31.25 pg/ml



Antibody Diluent Solution Concentrate – Reconstitute the Antibody Diluent Solution Concentrate with 11 mL of Dilution Buffer in provided 15 mL bottle to prepare Antibody Diluent Solution.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 105ul of Antibody Diluent Solution (DB20) to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Antibody Diluent Solution into a 15 ml centrifuge tube and transfer the 105 ul of 100-fold concentrated stock solution to prepare working solution. Note: Must be prepared 2 hours prior to use at room temperature. This is very important to control assay background.

Streptavidin-HRP Conjugate - Transfer 60u L of 200-fold concentrated stock solution to 11.94 mL of Dilution Buffer to prepare working solution. Note: 1X working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the positive control with 1.0 mL of Dilution Buffer to make positive control solution. Note: Positive Control should be used immediately.

ASSAY PROCEDURE

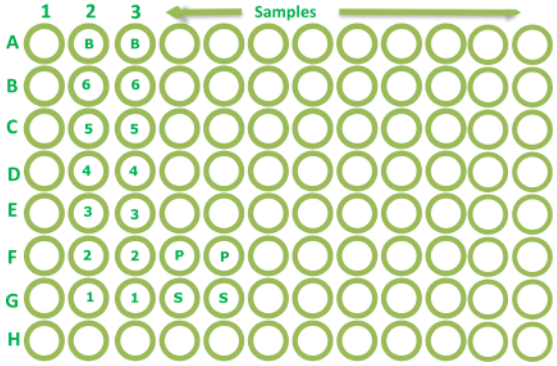
Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (A2, A3).
4. Add 100 µL of Standard (from B2 to G3, G4 to G5), sample, or positive control (F4 to F5) per well. Cover with the Sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 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 Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate** working solution to each well. Incubate for 1 hour on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 15-25 minutes at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log 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 sVEGF-R II 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.



CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human sVEGF-R II/Fc Chimera.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of sVEGF-R II was 15 pg/mL.

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.127)
31.25	0.048
62.5	0.096
125	0.181
250	0.364
500	0.682
1000	1.264
2000	2.274

*Lot No.:

** Positive Control: 54-90 pg/mL

LINEARITY

To assess the linearity of the assay, pooled research human **serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
20X	236.100	4722.00	100
40X	122.402	4896.08	103.7

SPECIFICITY

This assay recognizes both natural and recombinant human sVEGF-R II. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. No significant cross-reactivity or interference was observed.

PROTEIN	CROSS-REACTIVITY (%)
Human VEGF RII/Fc chimera	100
Human VEGF RI/Fc chimera	0.16%
Human VEGF-RIII/Fc	0
Human VEGF-D	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT. Prepare Detection Antibody working solution with Antibody Diluent Solution*.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin HRP conjugate working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate 15-25min on the bench top. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min

*Using Antibody Diluent Solution to prepare Detection Antibody working solution should be 2 hours prior to use.