HUMAN VEGF ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN VEGF CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN VEGF ELISA
Catalog No.	SK00122-01
Lot No.	
Formulation	96 T
Standard range	15.6-1000 pg/mL
Sensitivity	7.8 pg/mL
Sample Volume	100 µl
Sample Type	Serum, Plasma, Cell Culture Supernates
Dilution factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human VEGF
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2°C-8°C

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

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INTRODUCTION

Human VEGF immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human VEGF in cell culture supernates, serum and EDTA plasma. It contains recombinant Human VEGF and antibodies raised against this protein. It has been shown to accurately quantify recombinant human VEGF. Results obtained with naturally occurring VEGF 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 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, a biotinylated polyclonal antibody specific for VEGF is added to the wells. Following a wash to remove any unbound antibody, Streptavidin-HRP conjugate 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 VEGF 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
VEGF Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against VEGF.	**122-01-01**	**1 plate**
VEGF Standard – 2000 pg/vial of recombinant Human VEGF in a buffered protein base with preservatives; lyophilized.	**122-01-02**	**1 vial**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of polyclonal antibody against VEGF with preservatives; lyophilized.	**122-01-03**	**1 vial**
Positive Control - one vial of recombinant Human VEGF in a buffered protein base with preservatives; lyophilized.	**122-01-04**	**1 vial**
Streptavidin HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin HRP conjugate	**SAHRP**	**1 vial**
Dilution Buffer - 60 mL/bottle of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Wash Buffer - 50 ml/bottle, 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
Substrate Solution - 11 ml/bottle of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution – 11 mL/bottle of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard, Detection Antibody Solution SHOULD BE STORED at -20°C or – 70°C for up to one month. Streptavidi -HRP Conjugate 100-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. 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted Hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. 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.

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

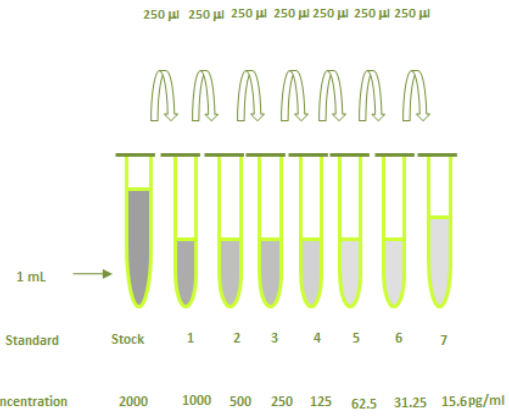
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes

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.

VEGF Standard - Refer to vial label for reconstitution volume. Reconstitute the **VEGF** Standard with 1 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 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 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
# 7	250 µl of 6	250 µl	15.625 pg/ml



Detection Antibody - Reconstitute the **Detection Antibody** with 105 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 105 µl of 100-fold concentrated stock solution to prepare working solution.

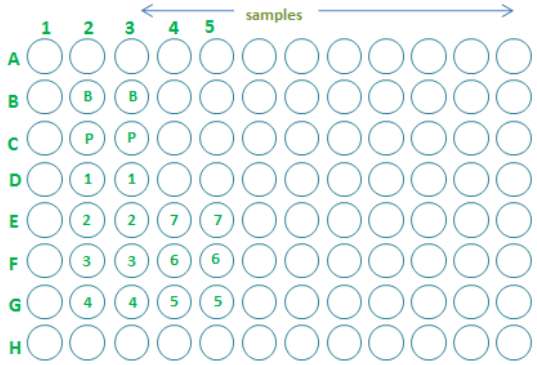
Streptavidin HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 11.88 mL of Dilution Buffer to prepare working solution. *Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.*

Positive Control - Reconstitute the positive control with 1mL of Dilution Buffer to make positive control solution.

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 bag containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (B2, B3).
4. Add 100 µL of Standard (D2, D3 to G2, G3, and E4, E5 to G4, G5), samples, or positive control (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on a microplate 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 30 minutes 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 3-8 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 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, 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 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.

VEGF (PG/ML)	CORRECTED (450NM)
Blank	0.095
15.6	0.021
31.25	0.035
62.5	0.077
125	0.166
250	0.371
500	0.715
1000	1.517

- **Lot No.:**
- **Positive Control : 190-370 pg/ml**

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human $VEGF_{165}$.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of VEGF was 2.5 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant Human VEGF. 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.

PROTEINS	CROSSREACTIVITY (%)
Human VEGF165	100
Human VEGF165b	100
Human VEGF121	100
Human $VEGF_{165}$/P1GF	23
Human P1GF	0
Human VEGF-C	0
Human VEGF-D	0
Mouse P1GF-2	0
Mouse $VEGF_{164}$	0
Rat $VEGF_{164}$	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl of standard, samples, positive control to each well. Incubate for 2 hours at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate for 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 hr 30 min on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 3-8 min on the bench top. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min