HUMAN SOLUBLE VEGFR2/CD309 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE VEGFR2 CONCENTRATIONS IN SERUM AND PLASMA.

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PURCHASE INFORMATION:
THIS IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN SOLUBLE VEGFR2 ELISA**
Catalog No.	**SK00123-08**
Formulation	**96 T**
Standard Range	**31 - 2000 pg/ml**
Sensitivity	**10 pg/mL**
Sample Volume	**100 µl per well**
Sample Type	**Serum, plasma**
Specificity	**Human Soluble VEGFR2**
Calibrator	**Human Soluble VEGF-R2 HEK293 derived**
Sample Dilution	**2-4**
Intra-assay Precision	**6-8%**
Inter-assay Precision	**8-12%**
Storage	**2 °C-8 °C for 1 month. More information see page 2-3**
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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INTRODUCTION

This Human soluble VEGFR2 ELISA Kit contains the necessary components required for the quantitative measurement of natural and recombinant human VEGFR2 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human VEGFR2 and antibody raised against this protein. Results from this immunoassay have shown to accurately quantify natural and recombinant human VEGFR2 samples.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human VEGFR2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human VEGFR2 present is bound by the immobilized antibody. After washing away any unbound substances, antibody HRP conjugate specific for human VEGFR2 is added to the wells. Following a wash to remove any unbound enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of human VEGFR2 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.
_ 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 the appropriate 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
Human VEGFR2 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against human VEGFR2	123-08-01	1 plate
VEGFR2 Standard – refer to lot specific of recombinant human VEGFR2 in a buffered protein base with preservatives; lyophilized.	123-08-02	1 vial
Detection Antibody HRP Conjugate –refer to lot specific concentrated of Antibody HRP conjugate against human VEGFR2 with preservatives;	123-08-03	1 vial
Dilution Buffer Concentrated- 8 mL/vial of 20-fold concentrated buffered protein based solution with preservatives	DB32	1 vial
Wash Buffer -25 ml/vial, 20-fold concentrated buffered surfactant, with preservative.	WB03	1 vial
Substrate Solution-11 ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution -11 ml /vial of 0.5M HCl	S-STOP	1 vial
Plate Sealer.	EAPS	1

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control, Dilution Buffer and Antibody Diluent Solution should be stored at -20 ˚C. Detection Antibody-HRP Conjugate concentrated solution should be stored only at 2 ~8 ° C. Substrate Solution can be stored at 2 – 8° C for up to 10 months. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may need a 2-fold ~ 4-fold dilution. A suggested 2-fold dilution is 125 µL sample + 125 µL 1x Dilution Buffer. A suggested 4-fold dilution is 60 µL sample + 180 µL 1x 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 bottle in a water bath until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer.

Standard – Reconstitute the standard refer to lot specific with of Dilution Buffer. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer.

STANDARD	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	Refer to lot specific	2000 pg/ml
# 1	Refer to lot		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

Detection Antibody-HRP Conjugate – refer to lot specific to prepare working solution. **Note:** 1x working solution of Detection IgG-HRP should be used within a few hours **(protect from light)**. **DO NOT FREEZE.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.
2. Remove unneeded microplate strips from the plate frame and return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of **Dilution Buffer** to Blank wells .
4. Add 100 µL per well of **Standard Dilutions** in reverse order of serial dilution , **sample,** or **positive control**. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.

5. Aspirate and wash each well with 300 µL of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
6. Add 100 µL per well of **Detection Antibody-HRP Conjugate working solution**. Cover with plate sealer and incubate for 2 hour on microplate shaker at room temperature. **Protect from light.**
7. Repeat the aspiration and wash as in step 5.
8. Add 100 µL per well of **Substrate Solution**. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
9. Add 100 µL per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Read plate using a microplate reader set to 450 nm within 5 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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 recombinant human VEGFR2, extracellular domain derived from HEK293.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human sVEGFR2 was 10 pg/mL.

TYPICAL DATA

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

STANDARD (PG/ML)	CORRECTED (450NM)
Blank	**0 (0.099)**
31.25	**0.039**
62.5	**0.081**
125	**0.156**
250	**0.322**
500	**0.648**
1000	**1.288**
2000	**2.145**

SPECIFICITY

PROTEINS	CROSS-REACTIVITY(%)
Human sVEGFR2	100
Human sVEGFR1	0
Human sVEGFR3	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS
↓
Add 100 µL of standard dilutions, samples and positive control. Cover with plate sealer and incubate 2 hours on microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate 2 hour on microplate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Substrate Solution. Incubate refer to lot on microplate shaker at RT. **Protect from light.**
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 5 minutes.