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Sunlong Medical™ Human VEGF ELISA Kit

Catalog Number: EL0047Hu
Size: 48 Test, 96 Test

Storage: 2-8℃
Validity Period: **Two Years**

Sensitivity:0.95 pg/mL
Assay range:31.25 pg/mL - 2000 pg/mL

For the **quantitative determination** of Vascular Endothelial Growth Factor (VEGF) concentrations **in cell culture supernates, serum and plasma**.

This package insert must be read entirely before using this product. For proper performance, follow the protocol provided with each individual kit.

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

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ASSAY PROCEDURE SUMMARY

1. Prepare all reagents and standards as directed.



2. Add 300 µl *Washing Bufer (1×)* per well to soak for about 30 seconds. Use immediately after aspirate.



3. Add100 µl 2-fold diluted *Standard* in duplicate. Add 100 µl *Standard Diluent/culture medium* to Blank well.



4. Serum/Plasma: Add 50 µl*AssayBufer (1×)* and 50 µl sample to the sample well.
Cell culture Supernates: Add 100 µl cell culture supernates to the sample well.



5. Add 50 µl of diluted *Detect Antibody* to each well. Step 3, 4 and 5 should be completed within 15 minutes. Incubate for 2 hours at RT. Aspirate and wash 6 times.



6. Add 100 µl of diluted Streptavidin-HRP to each well. Incubate for 45 minutes at RT. Aspirate and wash 6 times.



7. Add 100 µl *Substrate Solution* to each well.
Incubate for 5 - 30 minutes at RT. Protect from light.



8. Add 100 µl *Stop Solution* to each well.



9. Read at 450 nm within 30 minutes. Correction 570 or 630 nm.

DESCRIPTION

Vascular endothelial growth factor (VEGF) is a sub-family of growth factors, to be specific, the platelet-derived growth factor family of cystine-knot growth factors. They are important signaling proteins involved in both vasculogenesis (the de novo formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature). VEGF is a signal protein produced by cells that simulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. VEGF is best known for its role in vasculogenesis. During embryogenesis, VEGF regulates the proliferation, migration, and survival of endothelial cells, thus regulating blood vessel density and size, but playing no role in determining vascular patterns. Serum concentration of VEGF is high in bronchial asthma and diabetes mellitus. When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and metastasize. Overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body. Drugs such as bevacizumab and Ranibizumab can inhibit VEGF and control or slow those diseases.

PRINCIPLE OF THE ASSAY

Human VEGF ELISA Kit is based on the quantitative sandwich enzyme-linked immunosorbent assay technique to measure concentration of human VEGF in the samples. A monoclonal antibody specific for human VEGF has been immobilized onto microwells. Standard or samples are pipetted into the wells, followed by the addition of biotin-linked detect antibody specific for VEGF, and VEGF present is bound by the immobilized antibody and detect antibody following the first incubation. After removal of any unbound substances, streptavidin-HRP is added for a second incubation. After washing, substrate solution reacts with HRP and color develops in proportion to the amount of VEGF bound by the immobilized antibody. The color development is stopped by addition of acid and the optical density value is measured by microplate reader.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use expired kit or reagents.
- Do not use reagents from other lots or manufacturers. Do not prepare component by yourself.
- If concentration of assayed factor in samples is higher than the highest standard, dilute the serum/plasma samples with *Assay Bufer*, dilute the cell culture supernate samples with *cell culture medium*. Reanalyze these and multiply results by the appropriate dilution factor.
- Any variation in testing personnel, sample preparation, standard dilution, pipetting technique, washing techniques, incubation time, temperature, kit age and equipment can cause variation in results.
- This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED (96 Test)

Unopened kit should be stored at 2 - 8℃. 

- **Human VEGF Microplate** (1 plate): 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human VEGF.
- **Human VEGF Standard** (2 vials): Recombinant human VEGF in a buffered protein base with preservatives; lyophilized.
- **Human VEGF Detect Antibody** (1 vial): Biotin-conjugate anti-human VEGF detect antibody; 100× liquid.
- **Standard Diluent** (1 bottle, 5 ml): In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the Standard Diluent. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.
- **Streptavidin-HRP** (1 vial): 100× liquid.
- **Assay Buffer (10×)** (1 bottle, 5 ml): PBS with 0.5 % Tween-20 and 5 % BSA.
- **Substrate** (1 bottle, 11 ml): TMB (tetramethyl-benzidine).
- **Stop Solution** (1 bottle, 11 ml): 0.18 M sulfuric acid.
- **Washing Buffer (20×)** (1 bottle, 50 ml): PBS with 1 % Tween-20.
- **Plate Covers** (6 strips).

STORAGE

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

Unopened kit		Store at 2 - 8℃ (See expiration date on the label).
Opened/ Reconstituted Reagents	1× Washing Buffer 1× Assay Buffer Stop Solution Standard Diluent Substrate TMB Detect Antibody Streptavidin-HRP	Up to 1 month at 2 - 8℃.
	Standard	Up to 1 month at ≤ -20℃ in a manual defrost freezer. Discard after use.
	Microplate Wells	Up to 1 month at 2 - 8℃. Return unused strips to the foil pouch containing the desiccant pack, reseal along entire edge to maintain plate integrity.

Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- **Microplate reader** capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
- **Pipettes and pipette tips**.
- 50 µl to 300 µl adjustable **multichannel micropipette** with disposable tips.
- Multichannel micropipette **reservoir**.
- **Beakers, flasks, cylinders** necessary for preparation of reagents.
- **Deionized or distilled water**.
- **Polypropylene** test tubes for dilution.

PRECAUTION

- 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 material safety data sheet(s) and/or safety statement(s) for specific advice.
- The Stop Solution provided with this kit is an acid solution. Wear eyes, hand, face, and clothing protection when using this material.
- 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 and incubation.
- 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 HRP and antibody conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be warmed to 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℃.
- 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.
- In some cases, an insoluble precipitate of stabilizing protein has been seen in the Standard Diluent. This precipitate does not interfere in any way with the performance of the test and can thus be ignored. Or remove precipitate by centrifuging at 6,000 × g for 5 minutes.

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.
- When using an automated plate washer, adding a 30 seconds soak period before washing step and/or rotating the plate between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.
- It is recommended that all samples and standards be assayed in duplicate.
- Take care not to scratch the inner surface of the microwells.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Remove particulates by centrifugation at 300 × g for 10 minutes and assay immediately or aliquot and store samples at ≤ -20℃.

Serum – Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1,000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20℃.

Plasma – Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1,000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃.

Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay.

Note: 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℃ to avoid loss of bioactive human VEGF. If samples are to be run within 24 hours, they may be stored at 2 to 8℃.
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

REAGENT PREPARATION

Bring all reagents and samples to room temperature before use.

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1,000 ml graduated cylinder. Bring to final volume of 1,000 ml with pure or deionized water.

Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (5 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder. Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.

Store at 2 to 8℃. Assay Buffer (1×) is stable for 30 days.

Detect Antibody

Mix well prior to making dilutions.

Make a **1：100** dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1×) in a clean plastic tube as needed.

The diluted Detect Antibody should be used within 30 minutes after dilution.

Streptavidin-HRP

Mix well prior to making dilutions.

Make a **1：100** dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1×) in a clean plastic tube as needed.

The diluted Streptavidin-HRP should be used within 30 minutes after dilution.

Sample Dilution

If your samples have high VEGF content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

Human VEGF Standard

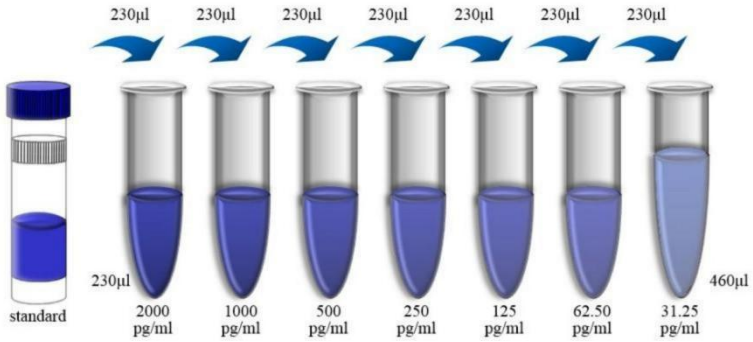
Reconstitute **Human VEGF Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 4,000 pg/ml).

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

Use polypropylene tubes.

For serum/plasma samples, mixing *concentrated human VEGF standard* (230 µl) with 230 µl of *Standard Diluent* creates the high standard (2,000 pg/ml). Pipette 230 µl of *Standard Diluent* into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. *Standard Diluent* serves as the zero standard (0 pg/ml).

For cell culture supernates, mixing *concentrated human VEGF standard* (230 µl) with 230 µl of cell culture medium creates the high standard (2,000 pg/ml). Pipette 230 µl of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ml).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.
2. Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.
3. **Soaking ELISA plate:** Add 300 µl *Washing Buffer (1 ×)* per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess *Washing Buffer (1 ×)*. Use the microwell strips immediately after washing. **Do not allow wells to dry.**
4. **Adding Standard:** Add 100 µl of 2-fold diluted *Standard* to Standard well in duplicate. Add 100 µl of *Standard Diluent* / *culture medium* o Blank well in duplicate.
5. **Adding Samples:** Serum/Plasma: Add 50 µl of *Assay Buffer (1 ×)* and 50 µl sample to the sample well. Cell culture supernates: Add 100 µl cell culture supernates to the sample well.
6. **Adding Detect Antibody:** Add 50 µl of **1:100** diluted *Detect Antibody* to each well. Ensure reagent addition in step 4, 5 and 6 is uninterrupted and completed within 15 minutes.
7. **Incubation:** Cover with an adhesive strip. Incubate at room temperature (25℃±3℃) for 2 hours on a microplate shaker set at 300 rpm.
8. **Washing:** Aspirate each well and wash, repeating the process five times for a total six washes. Wash by filling each well with 300µl *Washing Buffer (1 ×)*. 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.

9. **Adding Streptavidin-HRP:** Add 100 µl of **1:100** diluted *Streptavidin-HRP* to each well.
10. **Incubation:** Cover with a new adhesive strip. Incubate at room temperature (25℃±3℃) for 45 minutes on a microplate shaker set at 300 rpm.
11. **Washing:** Repeat aspiration/wash as in step 8.
12. **Adding Substrate Solution:** Add 100 µl of *Substrate Solution* to each well. Incubate for 5 - 30 minutes at room temperature. Protect from light.
13. **Stopping:** Add 100 µl of *Stop Solution* to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
14. **Readings:** Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

CALCULATION OF RESULTS

Average the duplicate optical density readings for each standards and sample, then subtract the average optical density value of the zero standard.

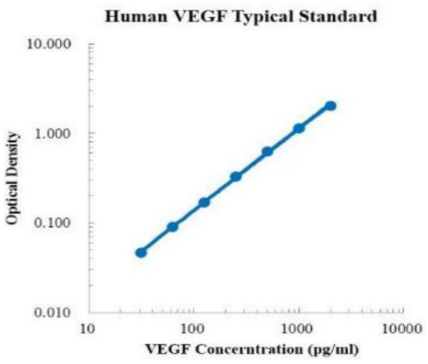
Standard Concentration as horizontal axis, optical density (OD) Value as the vertical axis, regressing the data and create a standard curve using computer software. The data may be linearized by plotting the log of the VEGF concentrations versus the log of the OD and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Note: The finally concentration of top standard is 2,000 pg/ml.
If samples have been diluted following the instruction, the final dilution factor is 2. If sample have been diluted by other means, the concentration read from the standard curve must be multiplied by the appropriate dilution factor.

TYPICAL DATA

A standard curve must be run within each assay. This standard curve is provided for demonstration only.

pg/ml	O.D.		Average	Corrected
0.00	0.026	0.027	0.027	
31.25	0.071	0.075	0.073	0.047
62.50	0.112	0.120	0.116	0.090
125.00	0.192	0.200	0.196	0.170
250.00	0.350	0.357	0.354	0.327
500.00	0.647	0.655	0.651	0.625
1000.00	1.155	1.176	1.166	1.139
2000.00	2.042	2.068	2.055	2.029



SENSITIVITY

The minimum detectable dose (MDD) of VEGF is typically about 0.95 pg/ml (mean of 6 independent assays).

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

PRECISION

Intra-assay Precision (Precision within an assay)

Three serum-based and buffer-based samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three serum-based and buffer-based samples of known concentration were tested in six separate assays to assess inter-assay precision.

	Intra-assay precision				Inter-assay precision		
Sample	1	2	3		1	2	3
n	20	20	20		6	6	6
Mean (pg/ml)	72.1	248.7	1024.5		70.4	250.8	1014.5
Standard deviation	2.4	11.2	46.1		3.0	13.8	69.0
CV (%)	3.3	4.5	4.5		4.2	5.5	6.8

RECOVERY

The spike recovery was evaluated by spiking 3 levels of human VEGF into five health human serum samples. The un-spiked serum was used as blank in these experiments.

The recovery ranged from 77 % to 119 % with an overall mean recovery of 100 %.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of VEGF in rat serum and diluted with Standard Diluent to produce samples with values within the dynamic range of the assay.

	Average (%)	Range (%)
1:2	89	85 - 112
1:4	92	85 - 99
1:8	95	86 - 103
1:16	94	87 - 105

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human VEGF produced at Sunlong Medical™.

SAMPLE VALUES

Serum/Plasma - Thirty samples from apparently healthy SD rats were evaluated for the presence of VEGF in this assay.

Sample Matrix	Number of Samples Evaluated	Range (pg/ml)	Detectable (%)	Mean of Detectable (pg/ml)
Serum	30	38.0 - 506.1	100	162.7

Note: The sample range is non-physiological range. The sample range of healthy rats will difference according to species, sample preparation, and testing personnel, equipment varies. The above information is only reference.

SPECIFICITY

This kit could assay both natural and recombinant human VEGF. A panel of substances listed below were prepared at 1 ng/ml in Standard Diluent to determine cross-reactivity. Preparations of the following substances at 1 ng/ml in a mid-range rhVEGF control to determine interference. No significant cross-reactivity or interference was observed.

Human		Mouse	Rat
IFN-γ	IL-17A	IFN-γ	IFN-γ
IL-1β	IL-18	IL-1β	IL-1β
IL-2	IL-21	IL-2	IL-4
IL-4	IL-22	IL-4	IL-6
IL-5	IL-23	IL-6	IL-10
IL-6	MCP-1	IL-10	IL-17A
IL-8	TGF-β1	IL-17A	TNF-α
IL-10	TNF-α	TNF-α	
IL-12			

PLATE LAYOUT

