

## Human VEGF ELISA Kit (hVEGF-ELISA)

*Cat. No. EK0539*

*96 Tests in 8 x 12 divisible strips*

### Background

Vascular endothelial growth factor (VEGF) is expressed in multiple cells and tissues including skeletal and cardiac muscle, hepatocytes, osteoblasts, neutrophils, macrophages, keratinocytes, brown adipose tissue, CD34<sup>+</sup> stem cells, endothelial cells, fibroblasts, and vascular smooth muscle cells. VEGF expression is induced by hypoxia and cytokines such as IL-1, IL-6, IL-8, oncostatin M and TNF- $\alpha$ . VEGF is expressed by most malignant tumors, has critical roles in vasculogenesis, and both physiological and pathological angiogenesis. VEGF produced by tumor cells potently stimulates endothelial cell proliferation and angiogenesis, and plays a key role in the pathophysiology of several neoplasias. In diseased tissues, VEGF promotes vascular permeability and contributes to tumor metastasis by promoting both extravasation and tumor angiogenesis. VEGF may also play a pivotal role in mediating the development and progression of diabetic retinopathy. VEGF binds to two receptor tyrosine kinases, KDR/Flk-1 and Flt-1. The VEGF gene is mapped by fluorescence in situ hybridization to chromosome 6p12.

ScienCell's human VEGF ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Human VEGF specific monoclonal capture antibodies are pre-coated onto 8 x 12 strips. The human-specific polyclonal detection antibodies are biotinylated. The test samples and biotinylated detection antibodies are subsequently added to the wells and then washed with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added next and unbound conjugates are washed away with PBS or TBS buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes to yellow after adding acidic stop solution. The intensity of yellow is proportional to the amount of human VEGF in the sample that is captured on the strips.

<b>Size</b>	96 Tests in 8 x 12 divisible strips
<b>Assay type</b>	Sandwich ELISA
<b>Range</b>	31.2pg/ml-2000pg/ml (human serum, body fluids) 15.6pg/ml-1000pg/ml (cell culture supernatants)
<b>Sensitivity</b>	< 1 pg/ml
<b>Specificity</b>	No detectable cross-reactivity with any other cytokine.
<b>Storage</b>	Store at 4°C for frequent use, at -20°C for infrequent use. Avoid multiple freeze-thaw cycles.
<b>Shipping</b>	Shipped on gel ice.

<b>Expiration</b>	Four months at 4°C and eight months at -20°C.
<b>Application</b>	For quantitative detection of human VEGF in serum, plasma, body fluids, tissue lysates or cell culture supernatants.
<b>Kit components</b>	<ol style="list-style-type: none"> <li>1. Lyophilized recombinant human VEGF standard: 10ng/tube×2.</li> <li>2. 8 x 12 divisible strips pre-coated with anti- human VEGF antibody.</li> <li>3. Sample diluent buffer: 30 ml</li> <li>4. Biotinylated anti- human VEGF antibody: 130µl, dilution 1:100.</li> <li>5. Antibody diluent buffer: 12ml.</li> <li>6. Avidin-Biotin-Peroxidase Complex (ABC): 130µl, dilution 1:100.</li> <li>7. ABC diluent buffer: 12ml.</li> <li>8. TMB color developing agent: 10ml.</li> <li>9. TMB stop solution: 10ml.</li> </ol>
<b>Materials</b>	1. Microplate reader.
<b>Required But Not Provided</b>	<ol style="list-style-type: none"> <li>2. Automated plate washer.</li> <li>3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended for large amount of samples.</li> <li>4. Clean tubes and Eppendorf tubes.</li> <li>5. Washing buffer (neutral PBS or TBS).</li> </ol> <p>Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H<sub>2</sub>O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.</p> <p>Preparation of 0.01 M PBS: Add 8.5g NaCl, 1.4g Na<sub>2</sub>HPO<sub>4</sub> and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.</p>
<b>Usage</b>	This product is for research use only. It is not approved for use in humans, animals, or <i>in vitro</i> diagnostic procedures.

## Reference

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5. Wei, M.-H.; Popescu, N. C.; Lerman, M. I.; Merrill, M. J.; Zimonjic, D. B. Localization of the human vascular endothelial growth factor gene, VEGF, at chromosome 6p12. *Hum. Genet.* 97: 794-797, 1996.

## Protocol for Human VEGF ELISA (96-well format)

### Notes before you begin

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color developing agent should be colorless and transparent before using.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. A duplicate well assay is recommended for both standard and samples.
5. Do not let wells dry, as this will inactivate active components in wells.
6. Do not reuse tips and tubes to avoid cross contamination.
7. Avoid using reagents from different batches.
8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before use.

### Preparation

#### Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C.

Avoid repeated freeze-thaw cycles.

- **Cell culture supernatant, tissue lysate or body fluids:** Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- **Serum:** Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 minutes. Analyze the serum immediately or aliquot and store frozen at -20°C.
- **Plasma:** Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at -20°C.

#### Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be mixed well with the diluent buffer.**

- **High target protein concentration (20-200ng/ml).** The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
- **Medium target protein concentration (2-20ng/ml).** The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
- **Low target protein concentration (31.2-2000pg/ml).** The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- **Very Low target protein concentration ( $\leq 31.2$ pg/ml).** No dilution necessary, or the working dilution is 1:2.

#### Reagent Preparation and Storage

A. Reconstitution of the human VEGF standard: VEGF standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of VEGF standard (10ng per tube) are included in each kit. Use one tube for each experiment.

- 10,000pg/ml of human VEGF standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 minutes and mix thoroughly.

- 2000pg/ml of human VEGF standard solution: Add 0.2 ml of the above 10ng/ml VEGF standard solution into 0.8 ml sample diluent buffer and mix thoroughly.
- 1000pg/ml→31.2pg/ml of human VEGF standard solutions: Label 6 Eppendorf tubes with 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 2000pg/ml VEGF standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

**Note:** The standard solutions are best used within 2 hours. The 10ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti- VEGF antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Biotinylated anti-human VEGF antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

### Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard VEGF detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of VEGF amount in samples.

1. Aliquot 0.1ml per well of the 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml human VEGF standard solutions into the pre-coated 8 x 12 divisible strips. Add 0.1ml of the sample diluent buffer into the control well (**blank well**). Add 0.1ml of each properly diluted sample of human serum, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. See “**Sample Dilution Guideline**” above for details. We recommend that each human VEGF standard solution and each sample is measured in duplicate.
2. Seal the strips with the cover and incubate at 37°C for 90 minutes.
3. Remove the cover, discard the strips’ contents, and blot the strips onto paper towels or other absorbent material. **Do NOT** let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human VEGF antibody working solution into each well and incubate the strips at 37°C for 60 minutes.
5. Wash the strips 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 minute. Discard the washing buffer and blot the strips onto paper towels or other absorbent material. (**Strips Washing Method:** Discard the solution in the wells without touching the side walls. Blot the strips onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of **THREE** washes. Note: For automated washing, aspirate all wells and wash **THREE** times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the strips onto paper towels or other absorbent material).
6. Add 0.1ml of prepared ABC working solution into each well and incubate the strips at 37°C for 30 minutes.

7. Wash the strips 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 minutes. Discard the washing buffer and blot the strips onto paper towels or other absorbent material.(See Step 5 for strip washing method).
8. Add 90 µl of prepared TMB color developing agent into each well and incubate the strips at 37°C in dark for 25-30 minutes (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human VEGF standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human VEGF concentration of the samples can be interpolated from the standard curve.

**Note:** if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

### Summary

1. Add samples and standards and incubate the strips at 37°C for 90 minutes. Do not wash.
2. Add biotinylated antibodies and incubate the strips at 37°C for 60 minutes. Wash strips 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the strips at 37°C for 30 minutes. Wash strips 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the strips at 37°C in dark for 25-30 minutes.
5. Add TMB stop solution and read.

### **Typical Data Obtained from Human VEGF**

(TMB reaction incubate at 37°C for 25 minutes)

Concentration (pg/ml)	0.0	31.2	62.5	125	250	500	1000	2000
Absorbance (450 nm)	0.036	0.142	0.231	0.374	0.663	1.134	1.817	2.318

### **Typical Human VEGF ELISA Kit Standard Curve**

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.

