



## Human VEGF ELISA

Catalog Number EA-0401

(For Research Use Only)

### Introduction

Vascular endothelial growth factor (VEGF) is one of the most potent and specific tumor-angiogenic factors (1, 2). VEGF is used by oxygen-hungry cells to promote growth of blood vessels. It binds to specialized receptors on the surfaces of endothelial cells and induces to build new vessels. Most tumors produce VEGF and inhibition of VEGF-induced angiogenesis significantly inhibits tumor growth in vivo (3-5). A number of antiangiogenic drugs have shown to be capable of decreasing the number of vessels. VEGF expression correlates with microvessel density in a number of solid malignancies including carcinomas of the breast, lung, prostate, and colon (6-9).

### Principle of the assay

VEGF ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes a mouse anti-human VEGF antibody for immobilization on the microtiter wells and goat anti-human VEGF antibodies along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the VEGF molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of VEGF is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

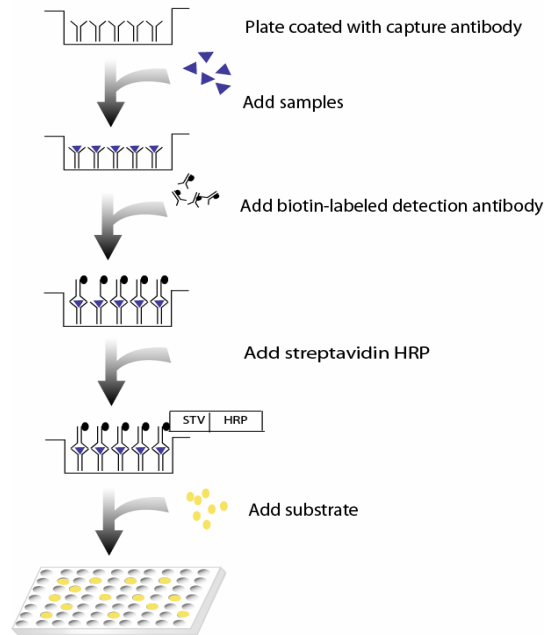


Diagram of ELISA

### Materials provided with the kit

- 96 well microplate coated with anti-human VEGF antibodies (4°C).
- Biotin labeled anti-human VEGF antibodies (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Recombinant VEGF standard (-20°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer (4°C).
- Substrate (4°C).
- Stop Solution (RT).

### Material required but not provided

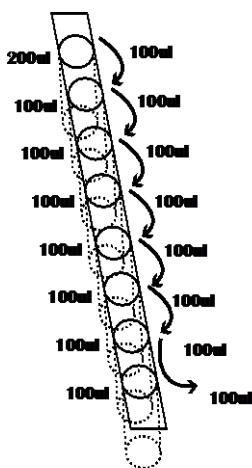
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

## Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer  
40ml 5x Assay wash buffer  
160ml ddH<sub>2</sub>O
- Dilute 100 times of human recombinant VEGF (400ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions. To dilute 100 times of human VEGF standard, add 2ul Human Recombinant VEGF in 200ul 1X Diluent Buffer (See Step 2 in "Assay Procedure" for detailed instruction
- Dilute 400 times of biotin labeled anti-human VEGF antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

## Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed
2. See instruction and diagram below for standard preparation.



- a. Add 200ul 1X Diluent buffer to the 1<sup>st</sup> well. Add 100ul 1X Diluent Buffer to the rest wells of strip.
- b. Add appropriate amount of protein recombinant (follow instruction in "Reagent Preparation")
- c. Mix dilutions in 1<sup>st</sup> well and transfer 100ul from the 1<sup>st</sup> well to the next dilution. (See picture) Incubate each well for 1 hr at room temperature with gentle shaking

3. Add 100ul of sample per well and incubate for 1 hour at room temperature with gentle shaking.
4. Aspirate each well and wash by adding 200ul of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 100ul of diluted biotin-labeled anti-human VEGF antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4.

7. Add 100 ul of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 4.
9. Add 100ul of substrate to each well and incubate for 10-30 minutes.
10. Add 50ul of Stop solution to each well. The color in the wells should change from blue to yellow.
11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

## Example of standard curve

