Signosis
Innovative Plate Assay Solutions

Rat VEGF ELISA

Catalog Number EA-3015 **(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 rabbit anti-rat VEGF antibodies for immobilization on the microtiter wells and rabbit anti-rat 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.

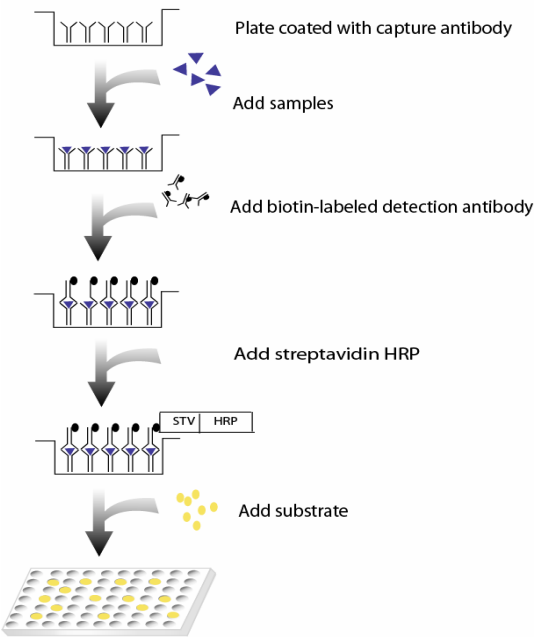


Diagram of ELISA

Materials provided with the kit

- 96 well microplate coated with rabbit anti-rat VEGF antibodies (4°C)
- Biotin labeled rabbit anti-rat VEGF antibodies (-20°C)
- Streptavidin-HRP conjugate (4°C)
- Rat recombinant VEGF standard (-20°C)
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer 4°C)
- Substrate (4°C)
- Stop Solution (4°C)

Material required but not provided

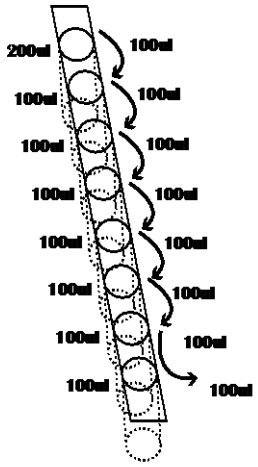
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 50 times of Rat recombinant VEGF (200ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions.
- To dilute 50 times of Rat recombinant VEGF, add 4ul Rat Recombinant VEGF in 200ul 1X Diluent Buffer (See Step 2 in "Assay Procedure" for detailed instruction
- Dilute 400 times of biotin labeled rabbit anti-rat VEGF antibody 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 1st 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 1st well and transfer 100ul from the 1st 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 200µl 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 100µl of diluted biotin-labeled rat anti-rat 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 µl 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 100µl of substrate to each well and incubate for 15-30 minutes.
10. Add 50µl 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