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VEGF (rat) ELISA Kit

rev 08/18

(Catalog # K5365-100, 100 assays; Store at -20^{0}C)

I. Introduction:

Vascular endothelial growth factor (VEGF) is a signaling messenger involved in endothelial cell proliferation and angiogenesis. BioVision's rat VEGF ELISA Kit is based on the standard principle of a sandwich enzyme-linked immunosorbent assay. A mouse monoclonal antibody specific for rat VEGF is coated on a 96-well plate. Standards and test samples are added to the wells and VEGF present in a sample is bound by the immobilized antibody. A biotinylated polyclonal antibody from goat specific for VEGF is added subsequently. After washing away the unbound biotinylated antibody with PBS or TBS buffer, avidin-Biotin-Peroxidase Complex is added to the wells. The wells are again washed with PBS or TBS buffer to remove the unbound conjugates. HRP substrate TMB is used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue product that changes into yellow after adding acidic stop solution. The density of yellow color is proportional to the rat VEGF captured onto the plate. This ELISA kit shows no detectable cross-reactivity with related proteins. Detection Range: 15.6 pg/ml – 1000 pg/ml. Sensitivity: < 1 pg/ml.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Native and recombinant rat VEGF

IV. Sample Type:

- Serum & plasma (EDTA)
- Cell culture supernatants, urine

V. Kit Contents:

Components	K5365-100	Part No.
Plate coated with anti-rat VEGF antibody, 96-wells	12 strips x 8 wells	K5365-100-1
Rat VEGF standard (10 ng/vial), lyophilized	2 vials	K5365-100-2
Biotinylated anti-rat VEGF antibody	130 µl	K5365-100-3
Avidin-Biotin-Peroxidase Complex (ABC)	130 µl	K5365-100-4
Sample diluent buffer	30 ml	K5365-100-5
Antibody diluent buffer	12 ml	K5365-100-6
ABC diluent buffer	12 ml	K5365-100-7
TMB color developing agent (Colorless)	10 ml	K5365-100-8
TMB stop solution	10 ml	K5365-100-9
Plate Sealer	4	K5365-100-10

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended for large number of samples.
- Washing buffer (neutral PBS or TBS).
  - Preparation of 0.01 M TBS: Add 1.2 g Tris, 8.5 g NaCl; 450 µl of purified acetic acid or 700 µl of concentrated hydrochloric acid to 1000 ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
  - Preparation of 0.01 M PBS: Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

VII. Storage Conditions and Reagent Preparation:

Store kit at 4^{o}C for 6 months or at -20^{o}C for 12 months. Avoid repeated freeze-thaw cycles. Centrifuge tubes briefly to spin down all components to the bottom before opening.

- **Reconstitution of the rat VEGF standard**: Two vials of VEGF standard (10 ng per vial) are included in each kit. Use one vial for each experiment. Prepare 10 ng/ml of rat VEGF standard solution by adding 1 ml of sample diluent buffer into one of the vials. Keep the tube at room temperature for 10 min. and mix thoroughly. Add 0.1 ml of the 10 ng/ml solution into 0.9 ml sample diluent buffer and mix thoroughly to make 1000 pg/ml stock. Label 6 Eppendorf tubes with 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml and 15,6 pg/ml respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the 1000 pg/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 hrs. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hrs, or at -20°C for up to 48 hrs. Avoid repeated freeze-thaw cycles.
- **Preparation of biotinylated anti-rat VEGF antibody working solution**: Dilute 1:100 with the antibody diluent buffer and mix thoroughly. Prepare 0.1 ml of antibody working solution for each well. Solution should be prepared no more than 2 hrs. prior to the experiment.
- **Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution**: Dilute 1:100 with the ABC dilution buffer and mix thoroughly. Prepare 0.1 ml of ABC working solution for each well. Solution should be prepared no more than 1 hr. prior to the experiment.

VIII. Sample Preparation and Storage:

- **Cell culture supernatants**: Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
- **Serum**: Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
- **Plasma**: Collect plasma using heparin, EDTA or citrate as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

Notes:

a. Store samples to be assayed within 24 hrs. at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

b. Sample dilution guidelines: 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 of the standard curve. Dilute the sample using the provided diluent buffer. The sample must be well mixed with the diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary to optimize sample dilution. For high target protein concentration (10-100 ng/ml): dilute 1:100. For medium target protein concentration (1-10 ng/ml): dilute 1:10. For low target protein concentration (15.6-1000 pg/ml): dilute 1:2. For very low target protein concentration (≤15.6 pg/ml). No dilution necessary or dilute 1:2.

IX. Assay Protocol:

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min. before use. When diluting samples and reagents, they must be mixed completely and evenly. The 96-well plate should not be dry at any time (drying will inactivate the active components on the plate).

1. Add 100 µl of the standard, samples, or control per well. At least two replicates of each standard, sample, or control is recommended.

   Notes: We recommend doing a pilot experiment using standards and a small number of samples to validate the assay procedure with the specific samples and optimize the appropriate sample dilution. Do not reuse tips and tubes to avoid cross contamination.

2. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
3. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
4. Add 0.1 ml of biotinylated anti-rat VEGF antibody working solution into each well and incubate the plate at 37°C for 60 min (or 90 min in RT).
5. Wash the plate 3 times with the 1x wash buffer. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash). Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 0.1 ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01 M TBS or 0.01 M PBS, and each time leave washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 3 for plate washing method).
7. Add 90µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 25-30 min.

   Note: These guidelines are for reference only; the optimal incubation time should be determined by end user. The shades of blue can be seen in the wells with the four most concentrated rat VEGF standard solutions; the other wells show no obvious color.

8. Add 0.1 ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
9. Read absorbance at 450 nm in a microplate reader within 30 min. after adding the stop solution.
10. Calculation: Relative O.D.450 = O.D.450 of each well – O.D.450 of Zero 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 rat VEGF concentration of the samples can be interpolated from the standard curve. Note: if the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the original concentration in the sample. Take the volume change due to activation of VEGF into account.

Typical Data Obtained from Rat VEGF

(TMB reaction incubated at 37°C for 25 min.)

Concentration(pg/ml)	0.0	15.6	31.2	62.5	125	250	500	1000
O.D	0.000	0.037	0.079	0.139	0.309	0.604	1.215	1.990

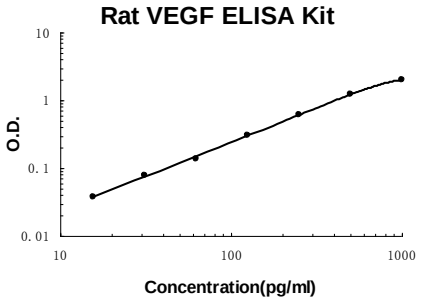

Figure: Standard Curve: This standard curves is for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

VEGF Antibodies (5363-100, 5363-100BFAF, 5364-100, 5365-100)
VEGF (mouse) ELISA Kit (K5364)
VEGF (human) ELISA Kit (K5363)
VEGF-C, Rat recombinant (4635)
VEGF165, Rat recombinant (4365)

FOR RESEARCH USE ONLY! Not to be used on humans.