

Rat VEGF ELISA Kit

Catalog No. CK448

Quantity: 1 x 96 tests

I. Introduction:

VEGF (Vascular endothelial growth factor) is called also VEGF-A, following the identification of several VEGF-related factors (VEGF-B, VEGF-C, VEGF-D, VEGF-E). VEGF significantly influences vascular permeability and is a strong angiogenic protein in several bioassays and probably also plays a role in neovascularization under physiological conditions. VEGF plays a role in the development and function of primate follicles and the ovarian corpus luteum, supporting the proliferation of blood vessels. The differentiation of adipocytes, of pheochromocytomas, and myocytes is accompanied by the controlled expression of VEGF. Recently, it has been demonstrated that inhibition of VEGF activity by treatment with a monoclonal antibody specific for VEGF can suppress tumor growth *in vivo*.

The Cell Sciences® Rat VEGF ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of rat VEGF in serum, plasma, and cell culture supernatants. This assay employs an antibody specific for rat VEGF coated on a 96-well plate. Standards and samples are pipetted into the wells and VEGF present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-rat VEGF antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of VEGF bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

II. Reagents:

- 1) VEGF Microplate (Item A): 96 well (12 strips x 8 wells) coated with anti-Rat VEGF.
- 2) Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution
- 3) Standards (Item C): 2 vials, recombinant Rat VEGF.
- 4) Assay Diluent A (Item D): 30 ml, 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.
- 5) Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For Standard/Sample (cell culture medium) diluent.
- 6) Detection Antibody VEGF (Item F): 2 vial of biotinylated anti-rat VEGF (each vial is enough to assay half microplate).
- 7) HRP-Streptavidin Concentrate (Item G): 8 µl 6000x concentrated HRP-conjugated streptavidin.
- 8) TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'- tetramethylbenzidine (TMB) in buffered solution.
- 9) Stop Solution (Item I): 8 ml of 2 M sulfuric acid.

III. Storage:

May be stored for up to 6 months at 2-4°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution. Opened Microplate Wells or reagents may be store for up to 1 month at 2-4°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles



Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 978 992-0298

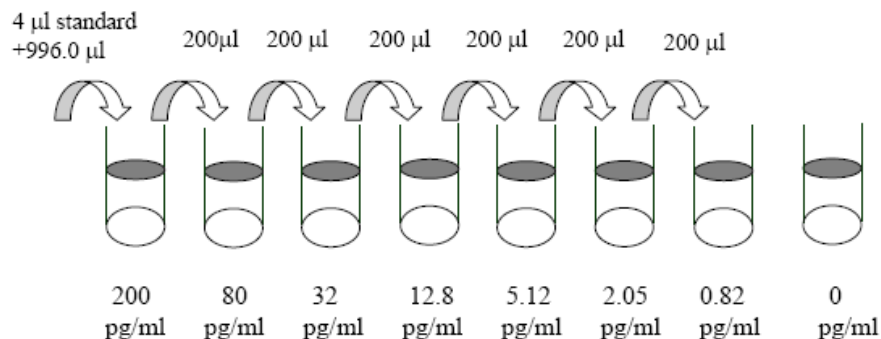
E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

IV. Additional Materials Required:

- 1) Microplate reader capable of measuring absorbance at 450 nm.
- 2) Precision pipettes to deliver 2 μ l to 1 ml volumes.
- 3) Adjustable 1-25 ml pipettes for reagent preparation.
- 4) 100 ml and 1 liter graduated cylinders.
- 5) Absorbent paper.
- 6) Distilled or deionized water.
- 7) Log-log graph paper or computer and software for ELISA data analysis.
- 8) Tubes to prepare standard or sample dilutions.

V. Reagent Preparation:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) is used for dilution of serum/plasma samples, and 1x Assay Diluent B (Item E) is used for dilution of culture supernatants.
3. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use.
4. Preparation of standard: **Briefly spin the vial of Item C** and then add 400 μ l Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium) into Item C vial to prepare a 50 ng/ml standard. **Dissolve the powder thoroughly by a gentle mix.** Add 4 μ l VEGF standard from the vial of Item C, into a tube with 996.0 μ l Assay Diluent A or 1x Assay Diluent B to prepare a 200 pg/ml stock standard solution. Pipette 300 μ l Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Gently vortex to mix. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).



5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 μ l of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 2-4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.



7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 6,000-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 2 µl of HRP-Streptavidin concentrate into a tube with 12 ml 1x Assay Diluent B to prepare a 6,000-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use).

VI. Assay Procedure:

- 1) Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
- 2) Add 100 µl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 2-4°C with gentle shaking.
- 3) Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. 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.
- 4) Add 100 µl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
- 5) Discard the solution. Repeat the wash as in step 3.
- 6) Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
- 7) Discard the solution. Repeat the wash as in step 3.
- 8) Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
- 9) Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. Assay Procedure Summary:

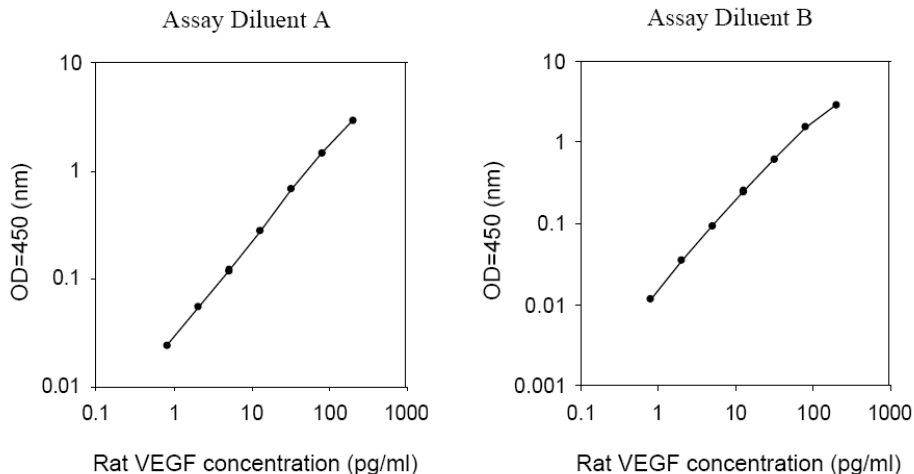
1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temp or overnight at 2-4°C.
3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.



VIII. Calculation of Results:

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data



Standard curves are for demonstration only. A standard curve must be run with each assay.

Sensitivity The minimum detectable dose of VEGF is typically less than 2 pg/ml.

Recovery Determined by spiking various levels of Rat VEGF into rat serum, plasma, and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	97.43	83-103
Plasma	96.42	85-104
Cell culture media	98.26	84-103

Linearity

Sample Type		Serum	Plasma	Cell culture media
1:2	Avg. % of Expected	96	95	96
	Range (%)	84-103	83-103	84-104
1:4	Avg. % of Expected	98	96	96
	Range (%)	86-104	84-104	84-103
1:8	Avg. % of Expected	95	97	101
	Range (%)	84-104	85-105	86-106

Reproducibility Intra-Assay: CV < 10%
Inter-Assay: CV < 12%



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Web Site: www.cellsciences.com

IX. Specificity:

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., rat CINC-2, CINC-3, CNTF, Fractalkine, IL-1 α , IL-1 β , IL-4, IL-6, IL-10, GM-CSF, IFN- γ , Leptin, Lix, MCP-1, MIP-3 α , β -NGF, TIMP-1, TNF- α).

X. Troubleshooting Guide:

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. Check pipettes 2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times 2. Inadequate reagent volumes or improper dilution	1. Ensure sufficient incubation time; assay procedure step 2 may change to over night 2. Check pipettes and ensure correct preparation
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
4. High background	1. Plate is insufficiently washed 2. Contaminated wash buffer	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed. 2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the ELISA kit 2. Stop solution	1. Store your standard at <-20°C after reconstitution, others at 4 °C. Keep substrate solution protected from light 2. Stop solution should be added to each well before measure

NOT FOR HUMAN USE. FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.



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