Product Manual

Revision date: 23/Feb/2017

Rat VEGF ELISA kit

Catalog No.: abx050233

Size: 96T

Range: 15.6 pg/ml - 1000 pg/ml

Sensitivity: < 1 pg/ml

Storage: Store at 2-8°C for 4 months, or at -20°C for 8 months.

Application: For quantitative detection of VEGF in Rat Serum, Cell Culture Supernatants, Plasma, Cell Lysates and other biological fluids.

Introduction: Vascular endothelial growth factor (VEGF), originally known as vascular permeability factor (VPF), is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate such as in hypoxic conditions. Serum concentration of VEGF is high in bronchial asthma and diabetes mellitus. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels.

Principle of the Assay

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Anti-VEGF is pre-coated onto 96-well plates. Biotin conjugated anti-VEGF antibody is used as a detection antibody. The standards, test samples and biotin conjugated antibody are added to the wells and washed with wash buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the VEGF amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of VEGF can be calculated.

Kit components

1. One 96 well plate.
2. Standard: 2 tubes
3. Sample / Standard diluent buffer: 30ml
4. Biotin conjugated antibody: 130µl - Dilution: 1:100
5. Antibody diluent buffer: 12 ml
6. Avidin-Biotin-Peroxidase Complex (ABC): 130 µl - Dilution: 1:100
7. ABC diluent buffer: 12 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 30 ml

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes to prepare standard/sample dilutions
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum at room temperature (~2 hr) or overnight at 4°C. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Cell culture supernatant:** Centrifuge at approximately 2000-3000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA as the anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Cell lysates:** Detach adherent cells with trypsin and collect by centrifugation and remove the supernatant. Wash the cells three times in ice-cold PBS and re-suspend cells in PBS. Lyse the cells by ultra-sonification 4 times or freeze at -20°C and thaw to room temperature 3 times. Centrifuge at 1500 × g for 10 min at 2-8°C to remove cellular debris. Collect the supernatant and assay immediately.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Samples must be diluted so that the expected concentration falls within the kit's range.
» Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.
» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.
» NaN3 cannot be used as test sample preservative, since it inhibits HRP.

General Sample guideline:

Estimate the concentration of the target protein in the sample and select the correct dilution factor to make the diluted target protein concentration fall near the middle of the kit's range. For high concentration, dilute 1:100, for medium concentration, dilute 1:10 and for low concentration, dilute 1:2. Very low concentrations do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

2. Wash buffer

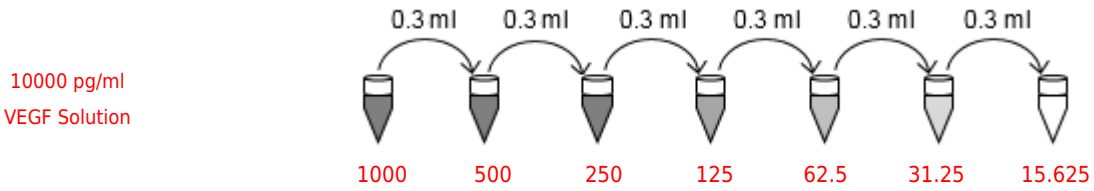
Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard

The standard solution should be prepared no more than 2 hours prior to the experiment. Note: Do not dilute the standard directly in the plate. Two tubes of standard are included in each kit. Use one tube for each experiment.

a.) 10,000 pg/ml of standard solution: Add 1 ml of Sample/Standard diluent buffer into one Standard tube to make the 10000 pg/ml solution. Keep the tube at room temperature for 10 min, mix thoroughly and avoid foaming or bubbles. Further dilute the above solution by a factor of 10 to give the highest standard (1000 pg/ml).

b.) 1000 pg/ml → 15.625 pg/ml standard solutions: Label 6 tubes with 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml and 15.625 pg/ml. Aliquot 0.3 ml of the Sample / Standard diluent buffer into each tube. Add 0.3 ml of the above 1000 pg/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.3 ml from the 1st tube to the 2nd tube and mix thoroughly. Transfer 0.3 ml from the 2nd tube to the 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. The standard solution should be used within 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

4. Preparation of Biotin conjugated antibody working solution: prepare no more than 2 hour before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the Biotin conjugated antibody with Antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of Antibody diluent buffer.

5. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: prepare no more than 1 hour before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the ABC with ABC diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of ABC into 99 µl of ABC diluent buffer.

B. Assay Procedure
Before adding to wells, equilibrate the ABC working solution and TMB substrate for at least 30 min at room temperature (18-25℃). It is recommend to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommend to measure each standard and sample in duplicate or triplicate.
2. Aliquot 0.1ml of the diluted standards into the standard wells.
3. Add 0.1 ml of Sample / Standard diluent buffer into the control (zero) well.
4. Add 0.1 ml of appropriately diluted sample into test sample wells.
5. Seal the plate with a cover and incubate at 37℃ for 90 min.
6. Remove the cover and discard the plate content, tap the plate on absorbent filter papers. **Do not let the wells completely dry at any time. Do not wash plate.**
7. Add 0.1 ml of Biotin conjugated antibody working solution into the wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37℃ for 60 min.
9. Remove the cover and wash the plate 3 times with Wash buffer using one of the following methods:

Manual Washing: Discard the solution without touching the side walls. Tap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer and incubate on an ELISA shaker for 2 min. Discard the contents and tap the plate on absorbent filter papers or other absorbent material. Repeat this procedure three times.

Automated Washing: Discard the solution and wash the plate three times with Wash buffer (overfilling wells with the buffer). After the final wash tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a

soaking time of 1 min.

10. Add 0.1 ml of ABC working solution into each well, cover the plate and incubate at 37℃ for 30 min.
11. Remove the cover and wash the plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1-2 min. (See Step 9 for plate wash method).
12. Add 0.1 ml of TMB substrate into each well, cover the plate and incubate at 37℃ in dark conditions for 15-30 min. (Note: This incubation time is for reference only, the optimal time should be determined by the end user.)
13. Add 0.1 ml of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
14. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450nm immediately.

For calculation: (the relative O.D.450) = (the O.D.450 of each well) – (the 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). Log-log curve fitting is recommended for data analysis. The Rat VEGF concentration of the samples can be interpolated from the standard curve.
Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.Equilibrate the ABC working solution for at least 30 minutes to room temperature prior to use. It is recommended to plot a standard curve for each test.

C. Precautions

1. Before the experiment, centrifuge the tubes briefly to bring down any contents trapped in the lid.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. It is recommend to assay all standards, controls and samples in duplicate or triplicate.
4. Do NOT let the plate completely dry at any time as this will inactivate the biological material on the plate
5. Ensure plates are properly sealed or covered during incubation steps.
6. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
7. Do not reuse pipette tips and tubes to avoid cross contamination.
8. Do not use expired components or components from different batches.
9. Store the TMB substrate in the dark and to avoid the edge effect of plate incubation for temperature differences it is recommend to equilibrate the ABC working solution and TMB substrate for at least 30 min at room temperature (18-25℃) before adding to wells.
10. The TMB substrate is colorless and transparent before use, if not, please contact us for replacement.

D. Typical Data & Standard Curve
Results of a typical standard run of a Rat VEGF ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.

Concentration pg/ml	0	15.625	31.25	62.5	125	250	500	1000
OD450	0.064	0.101	0.143	0.211	0.363	0.685	1.231	2.374