Catalog Number	Size
OKAA00029_96W	96 Tests
OKAA00029_48W	48 Tests

Human VEGF ELISA KIT

For the quantitative determination of human vascular endothelial growth factor (VEGF) concentrations in cell culture supernates, serum, and plasma.This package insert must be read in its entirety before using this product. If you have questions or experience problems with this product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

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INTRODUCTION

Vascular endothelial growth factor (VEGF or VEGF-A), also known as vascular permeability factor (VPF), is well known for its important roles in regulating both physiological and pathological blood vessel growth (1). It is a member of the VEGF family that also includes VEGF-B, -C, -D, -E, and P*l*GF (placental growth factor) (2). Family members exhibit a cysteine knot motif formed by eight characteristically spaced cysteine residues (3 - 5).

Various cells and tissues express VEGF including skeletal and cardiac muscle, hepatocytes, osteoblasts, neutrophils, macrophages, keratinocytes, platelets, brown adipose tissue, CD34+ cells, astrocytes, neurons, and endothelial cells (6-17). VEGF can be detected in both human plasma and serum samples, with higher levels found in serum due to its release from platelets (17). VEGF transcription is potentiated in response to hypoxia, oncogenic transformation, and growth factors.

VEGF has several physiological roles both during development and in the adult. It has a well-documented and critical role in embryonic vasculogenesis (1). Targeted deletion of a single VEGF allele results in significant defects in vascular development and is embryonic lethal (18). VEGF acts as an endothelial cell survival factor, mitogen, and inducer of migration, functions that potentially involve an array of signaling molecules including G proteins, PI3 kinase, MAP kinases, FAK, PKC, and NO (3, 19-25). VEGF affects bone formation by regulating blood vessel growth and cartilage remodeling, activities important for growth plate morphogenesis (26). In the adult, VEGF has potential roles in cyclic angiogenesis in the female reproductive system. Other diverse functions include the regulation of hematopoietic development and roles as a neurotrophic factor.

VEGF has received much attention for its involvement in tumor-associated angiogenesis and its potential as a target for cancer therapy. Numerous studies have demonstrated that VEGF or its receptors are upregulated in several forms of human cancer. Neutralizing VEGF antibodies are shown to reduce tumor growth in cancer models.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for VEGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any VEGF present is bound by the immobilized antibody. Following

incubation unbound samples are removed during a wash step, and then a detection antibody specific for VEGF is added to the wells and binds to the combination of capture antibody- VEGF in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A colored product is formed in proportion to the amount of VEGF present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven VEGF standard dilutions and VEGF sample concentration determined.

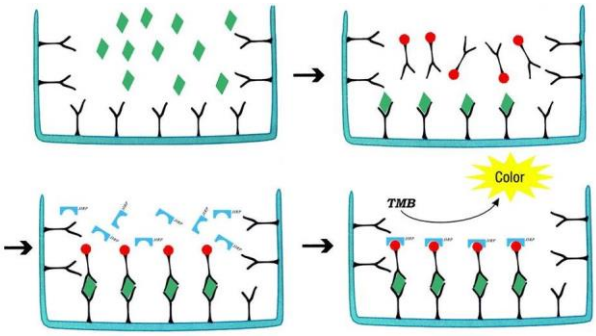

Figure 1: Schematic diagram of the assay

REAGENTS

1. Aluminum pouches with a Microwell Plate coated with antibody to human VEGF (8×6)
2. 1 vial human VEGF Standard lyophilized, 2000 pg/ml upon reconstitution
3. 1 vial concentrated Biotin-Conjugate anti-human VEGF antibody
4. 1 vial Streptavidin-HRP solution
5. 1 bottle Standard /sample Diluent
6. 1 bottle Biotin-Conjugate antibody Diluent
7. 1 bottle Streptavidin-HRP Diluent
8. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)
9. 1 vial Substrate Solution
10. 1 vial Stop Solution

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11. 2 pieces Adhesive Films

12. package insert

NOTE: [48 Tests]

STORAGE

Table 1: Storage of the kit

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Standard /sample Diluent</td><td rowspan="8">May be stored for up to 1 month at 2 - 8° C.**</td></tr>
<tr><td>Concentrated Biotin-Conjugate</td></tr>
<tr><td>Streptavidin-HRP solution</td></tr>
<tr><td>Biotin-Conjugate antibody Diluent</td></tr>
<tr><td>Streptavidin-HRP Diluent</td></tr>
<tr><td>Wash Buffer Concentrate 20x</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20°C.
Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.**</td></tr>
</table>

**Provided this is within the expiration date of the kit.

THE REQUIRED ITEMS (not provided, but can help to buy):

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000ul.
3. 37 ° C incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

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PRECAUTIONS FOR USE

1. Store kit reagents between 2° C and 8° C. After use all reagents should be immediately returned to cold storage (2° C to 8° C).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

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SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).
   Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard** - Reconstitute the Standard with 1.0mL of Standard /sample Diluent. This reconstitution produces a stock solution of 2000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Pipette 500μL of Standard/sample Diluent into the 1000 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 2000 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

   If you do not run out of re-melting standard, store it at -20° C. Diluted standard shall not be reused.

4. Working solution of Biotin-Conjugate anti-human VEGF antibody: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

5. Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

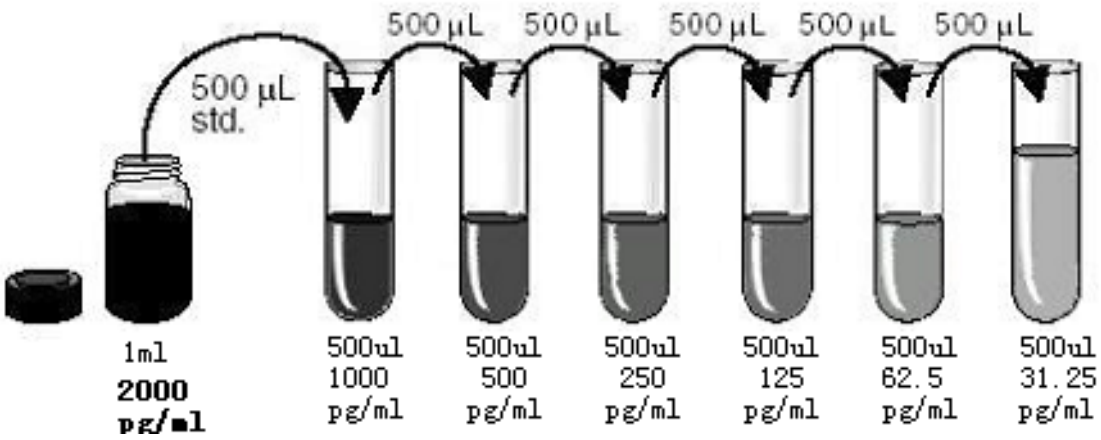

Figure 2: Preparation of VEGF standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8° C sealed tightly.
3. Add 100µL of Standard, control, or sample, per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at 37° C.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350µL) using a squirt bottle, manifold dispenser or auto-washer. 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.

5. Add 100 μL of the working solution of Biotin-Conjugate to each well. Cover with a new adhesive strip and incubate 1 hour at 37° C.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μL of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at 37° C Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μL of Substrate Solution to each well. Incubate for 10-20 minutes at 37° C. Avoid placing the plate in direct light.
10. Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.(optionally 630nm as the reference wave length; 610-650nm is acceptable)

ASSAY PROCEDURE SUMMARY

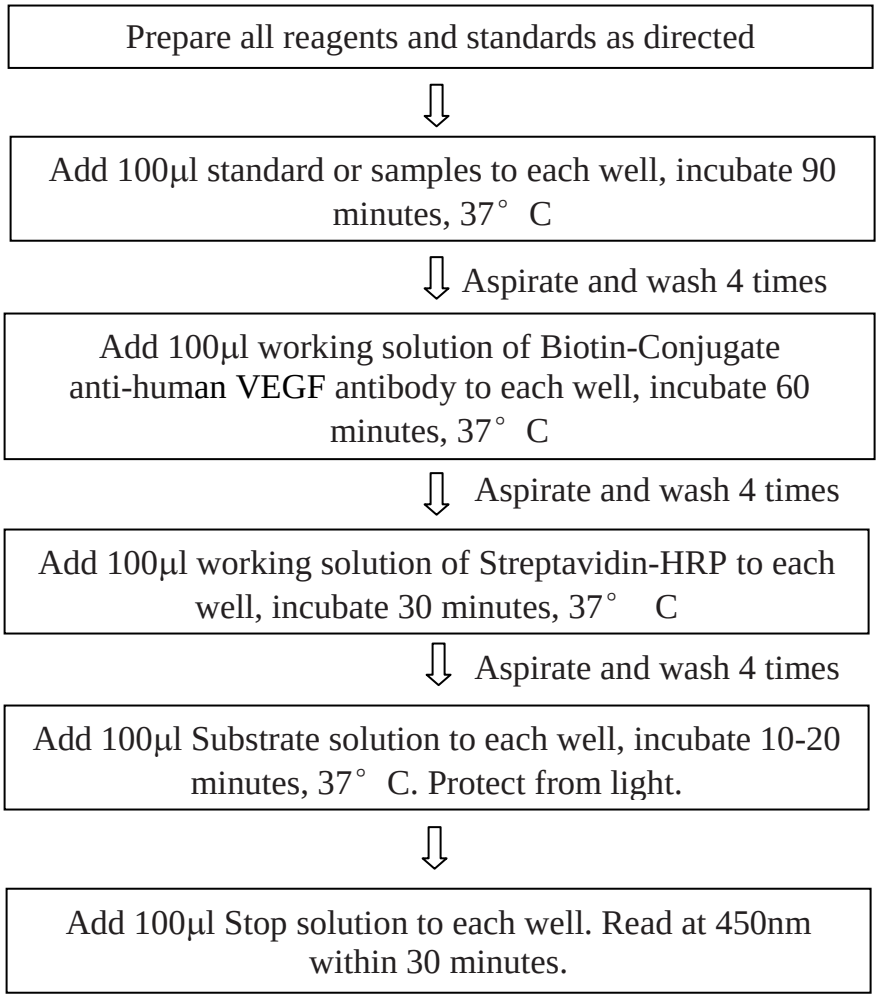

Figure 3: Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

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3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
3. The data may be linearized by plotting the log of the VEGF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 2: Typical data using the VEGF ELISA (Measuring wavelength: 450nm, Reference wavelength: 630nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.049	0.043	0.046	——
31.25	0.154	0.152	0.153	0.185
62.5	0.256	0.255	0.255	0.261
125	0.458	0.460	0.459	0.409
250	0.734	0.732	0.733	0.687
500	1.252	1.250	1.251	1.176
1000	1.801	1.804	1.802	1.886
2000	2.246	2. 244	2.245	2.230

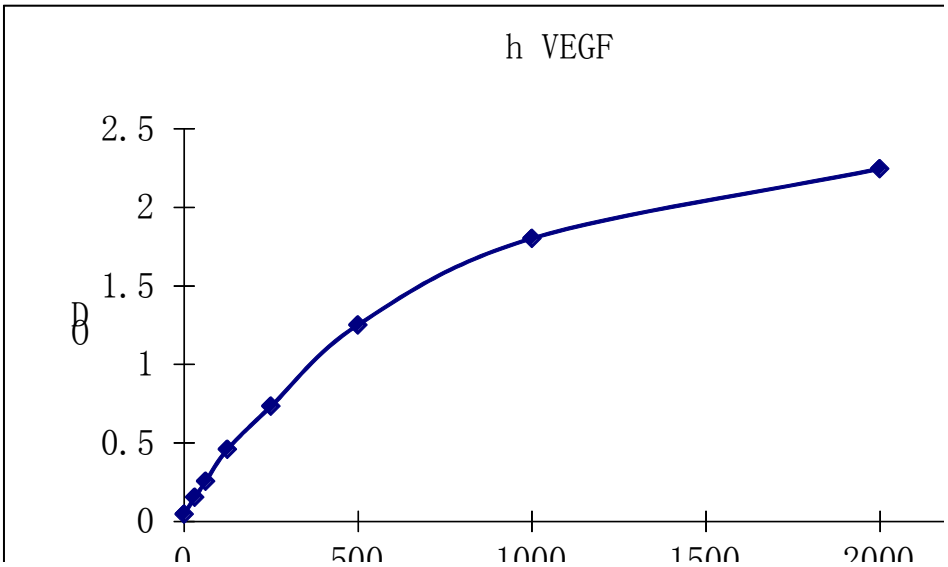

Figure 4: Representative standard curve for VEGF ELISA. VEGF was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

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SENSITIVITY, SPECIFICITY AND REPEATABILITY

1. **REPEATABILITY**: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
2. **SENSITIVITY**: The minimum detectable dose was 7pg/mL.
3. **SPECIFICITY:** This assay recognizes both natural and recombinant human VEGF. The factors listed below were prepared at 200 ng/ml in Standard/ sample diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 3: Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant Rat
EC-VEGF/PK1	PlGF-2	VEGF 120(0.07%)
PDGF-AA	VEGF120	
PDGF-AB	VEGF164	
PDGF-BB	VEGF-D	
PlGF	VEGF R2(KDR)/Fc Chimera	

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RELATED PRODUCTS

Table 3: Related products

Product Name	Catalog Number	Size
human EGF ELISA kit	OKAA00001	48T/96T
human Fas ELISA Kit	OKAA00002	48T/96T
human G-CSF ELISA kit	OKAA00003	48T/96T
human GDF-15 ELISA kit	OKAA00004	48T/96T
human GM-CSF ELISA kit	OKAA00005	48T/96T
human IFN-r ELISA kit	OKAA00006	48T/96T
human IL-1a ELISA kit	OKAA00007	48T/96T
human IL-2sRa ELISA kit	OKAA00008	48T/96T
human IL-3 ELISA kit	OKAA00009	48T/96T
human IL-4 ELISA kit	OKAA00010	48T/96T
human IL-5 ELISA kit	OKAA00011	48T/96T
human IL-6 ELISA kit	OKAA00012	48T/96T
human IL-9 ELISA kit	OKAA00013	48T/96T
human IL-10 ELISA kit	OKAA00014	48T/96T
human IL-12p40 ELISA kit	OKAA00015	48T/96T
human IL-12p70 ELISA kit	OKAA00016	48T/96T
human IL-15 ELISA kit	OKAA00017	48T/96T
human IL-17 ELISA kit	OKAA00018	48T/96T
human IL-21 ELISA kit	OKAA00019	48T/96T
human IL-22 ELISA kit	OKAA00020	48T/96T
human IL-32a ELISA kit	OKAA00021	48T/96T
human Leptin ELISA kit	OKAA00022	48T/96T
human MCP-1 ELISA kit	OKAA00023	48T/96T
human MMP-9 ELISA kit	OKAA00024	48T/96T
human sICAM-1 ELISA kit	OKAA00025	48T/96T
human TGF-b1 ELISA kit	OKAA00026	48T/96T
human TNF-a ELISA kit	OKAA00027	48T/96T
human TSLP ELISA kit	OKAA00028	48T/96T
human VEGF ELISA kit	OKAA00029	48T/96T
human FGF-Basic ELISA kit	OKAA00030	48T/96T
Human IL-2 ELISA kit	OKAA00031	48T/96T
Human IL-8 ELISA kit	OKAA00032	48T/96T