Code No. 27171

Human VEGF Assay Kit - IBL

INTRODUCTION

Vascular Endothelial Cell Growth Factor (VEGF) is a homodimeric protein initially purified from media conditioned by normal bovine pituitary folliculo-stellate cells and secreted by a variety of vascularized tissues. It was subsequently found to be identical to a vascular permeability factor (VPF), which was previously identified in media conditioned by tumor cell lines based upon its ability to increase the permeability of capillary blood vessels. The reported activities of VEGF include stimulation of endothelial cell growth, angiogenesis and capillary permeability. Human VEGF is a 38.2kDa homodimeric protein consisting of two 165 amino acid polypeptide chains. VEGF is expressed in many human tumor cells, including human adenocarcinoma, human pancreatic carcinoma, human hepatocellular carcinoma, renal cell carcinoma, fibrosarcoma, HL60 promyelocytic leukemia, GS-9L glioma and U937 lymphoma cells. In normal tissues, VEGF expression has been observed in activated macrophages, keratinocytes, hepatocytes, smooth muscle cells Leydig cells, embryonic fibroblasts and bronchial and choroids plexus epithelium, renal glomerular visceral epithelium and mesangial cells.
The IBL's Human VEGF ELISA kit is a complete kit for the quantitative determination of human VEGF in EDTA plasma and supernatant of cell culture media.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as coloring agent (Chromogen). The strength of coloring is in proportion to the quantities of Human VEGF.

MEASUREMENT RANGE

15.63 ～ 1,000 pg/mL (1st. incubation for 60 minutes at 37℃)
7.81 ～ 500 pg/mL (1st. incubation overnight at 4℃)

INTENDED USE

For research use only, not for use in diagnostic procedures.

This kit is to be used for the in-vitro quantitative determination of Human VEGF in EDTA plasma or cell culture media. The assay will recognize both natural and recombinant Human VEGF. This kit is a solid phase sandwich ELISA using high specific polyclonal antibody and monoclonal antibody.

KIT COMPONENT

1	Precoated plate : Anti-Human VEGF (16F1) Mouse IgG MoAb Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti-Human VEGF-1 Rabbit IgG Fab' Affinity Purify	0.4mL x 1
3	Standard : Recombinant Human VEGF 165	0.5mL x 2
4	EIA buffer*	30mL x 1
5	Solution for Labeled antibody*	12mL x 1
6	Chromogen: TMB solution	15mL x 1
7	Stop solution*	12mL x 1
8	Wash buffer Conc.*	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Incubator (37℃±1℃)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Refrigerator (as 4℃)
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"

2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. The temperature of "8, Wash buffer Conc." shall be adjusted to room temperature and then, mix it gently and completely before use. Dilute 50mL of "8, Wash buffer Conc." with 1,950mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.

2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one slit (8 well), the required quantity of Labeled antibody is 800 μL. (Dilute 30 μL of "2, Labeled antibody Conc." with 870 μL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 μL in each well.)
<u>This operation should be done just before the application of Labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4℃ in firmly sealed vial.

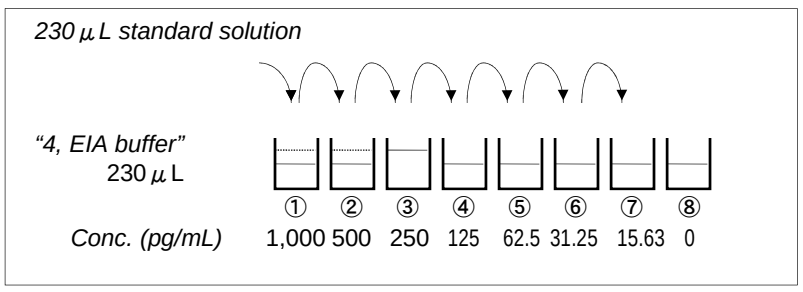
3) Preparation of Standard
Put just <u>0.5 mL</u> of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 2,000 pg/mL Human VEGF standard. The standards enclosed in this kit can be frozen and stored after reconstitution. However the freeze-thaw shall not be repeated.

4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 μL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube.

Tube-1	1,000 pg/mL	
Tube-2	500 pg/mL	
Tube-3	250 pg/mL	
Tube-4	125 pg/mL	
Tube-5	62.5 pg/mL	
Tube-6	31.25 pg/mL	
Tube-7	15.63 pg/mL	
Tube-8	0 pg/mL	(Test Sample Blank)

Put 230 μL of Standard solution into tube-1 and mix it gently. Then, put 230 μL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 1,000 pg/mL and 15.63 pg/mL. Tube-8 is the test sample blank as 0 pg/mL.

See following picture.



5) Dilution of test sample
Test sample may be diluted with "4, EIA buffer" if the need arises.
If the concentration of Human VEGF in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Confirm no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 μL	Diluted standard (Tube 1~7) 100 μL	EIA buffer (Tube-8) 100 μL	EIA buffer 100 μL
Incubation for 60 minutes at 37℃ or overnight at 4℃ with plate lid				
4 times (wash buffer more than 350 μL)				
Labeled Antibody	100 μL	100 μL	100 μL	-
Incubation for 30 minutes at 4℃ with plate lid				
5 times (wash buffer more than 350 μL)				
Chromogen	100 μL	100 μL	100 μL	100 μL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 μL	100 μL	100 μL	100 μL
Read the plate at 450nm against a Reagent Blank within 30 minutes after application of Stop solution.				

1) Determine wells for reagent blank. Put 100 μL each of "4, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 μL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1~7) into the appropriate wells.
3) Incubate the precoated plate for 60 minutes at 37℃ or overnight at 4℃ after covering it with plate lid.
4) Wash the plate with the prepared wash buffer and remove all liquid.
5) Pipette 100 μL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 4℃ after covering it with plate lid.
7) Wash the plate with the prepared wash buffer and remove all liquid.
8) Take the required quantity of "6, Chromogen" and put it into a disposable test tube. Then, pipette 100 μL from the test tube into every well. Please do not return the rest of used chromogen in the test tube into "6, Chromogen" bottle in order to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by the addition of "6, Chromogen".
10) Pipette 100 μL of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by the addition of "7, Stop solution".
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450nm.
The measurement shall be done within 30minutes after the addition of "7, Stop solution".

SPECIAL ATTENTION

1) Test samples should be measured soon after the collection. In case of the storage of test samples, they should be stored under frozen conditions and do not repeat freeze/thaw cycles. Thaw the test samples at low temperature and mix them completely before measurement.
2) Test samples should be diluted with "4, EIA buffer", suitably.

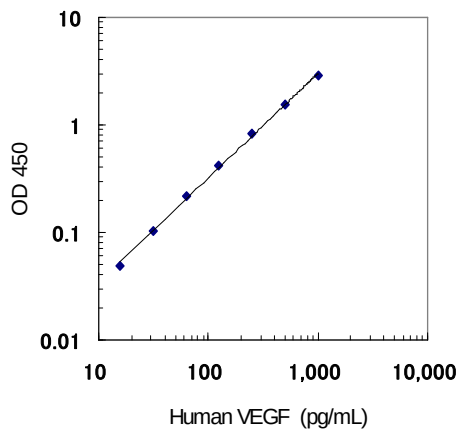
3) The measurement of test samples and standard in duplicate is recommended.
4) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5) Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
6) Remove the wash buffer completely by tapping the precoated plate on paper towel.
   Do not wipe wells with paper towel.
7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
8) Measurement should be done within 30 minutes after addition of "7, Stop solution".
9) The plasma sample collected by Heparin gives rather low data compare to assay by EDTA plasma.

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve (1st. incubation for 60 minutes at 37℃)

Conc. (pg/mL)	Absorbance (450nm)
1000	2.855
500	1.544
250	0.836
125	0.433
62.5	0.236
31.25	0.121
15.63	0.068
0 (Test Sample Blank)	0.018



* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Titer Assay (Samples with standard added are used.)

Specimen	Titer (X)	Measurement Value (pg/mL)	Theoretical Value (pg/mL)	%
Medium with 10% FBS	4	256.97	250.93	102.4
	8	113.30	125.00	90.6
	16	59.71	63.43	94.1
	32	30.28	31.85	95.1
Human plasma (EDTA)	8	280.71	251.88	111.4
	16	119.18	125.00	95.3
	32	57.69	62.50	92.3
	64	28.46	31.25	91.1

2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
Medium with 10% FBS (x4)	250.54	264.72	105.7
	125.54	120.31	95.8
	63.04	58.80	93.3
	31.79	30.05	94.5
Human plasma (EDTA) (x16)	252.38	202.64	80.3
	127.38	90.52	71.1
	64.88	46.08	71.0
	33.63	23.27	69.2

3. Intra - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
346.46	19.68	5.7	26
88.22	5.23	5.9	26
22.96	1.30	5.7	26

4. Inter - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
320.19	19.32	6.0	4
78.10	7.10	9.1	4
22.09	2.32	10.5	4

5. Specificity

Compound	Cross Reactivity
Human VEGF 165	100.0%
Human VEGF 121	39.7%
Human PDGF	< 0.1%

6. Sensitivity

1.01 pg/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2~8℃. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. "3, Standard" is lyophilized products. Be careful to open this vial.
3. "7, Stop solution" is a strong acid substance. Therefore, be careful not to contact your skin and clothes with "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in "2, Labeled antibody Conc.", "4, EIA buffer" or "8, Wash buffer Conc.", however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from different lot or different kit.
8. Do not use the reagents expired.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8 °C
The expiry date is specified on outer box.

REFERENCE

1. Hirata C, Nakano K, Nakamura N, Kitagawa Y, Shigeta H, Hasegawa G, Ogata M, Ikeda T, Sawa H, Nakamura K, Ienaga K, Obayashi H, Kondo M. Advanced glycation End Products Induce Expression of Vascular Endothelial Growth Factor by Retinal Muller Cells. Biochem Biophys Res Commun. 1997 Jul 30;236 (3):712-5.
2. Yamamoto S, Konishi I, Mandai M, Kuroda H, Komatsu T, Nanbu K, Sakahara H and Mori T. Expression of vascular endothelial growth factor (VEGF) in epithelial ovarian neoplasms: correlation with clinicopathology and patient survival, and analysis of serum VEGF levels. Br J Cancer. 1997;76 (9):1221-7.
3. Shishido T, Yasoshima T, Denno R, Mukaiya M, Sato N, Hirata K. Inhibition of liver metastasis of human pancreatic carcinoma by angiogenesis inhibitor TNP-470 in combination with cisplatin. Jpn J Cancer Res. 1998 Sep;89 (9):963-9.

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