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AuthentiKine™

Human VEGF Sandwich ELISA Kit Datasheet

For the quantitative detection of Human VEGF in serum, plasma and cell culture supernatants samples.

General Information

Catalogue Number	KE00216
Product Name	AuthentiKine™ Human VEGF Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	7422
SwissProt	P15692

Kit Components & Storage

Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 µL/vial	1 vial	
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 4B1 - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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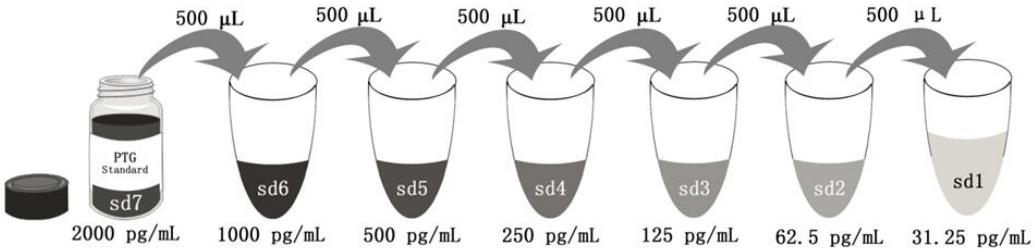
NB: Do not use the kit after the expiration date.
Sample Diluent PT **4B1** is for protein standard and samples.
Detection Diluent is for Detection antibody and HRP-conjugated antibody.
*Add **2 mL** Sample Diluent PT 4B1 in protein standard. This reconstitution gives a stock solution of 2000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00216 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The VEGF ELISA kit is to be used to detect and quantify protein levels of endogenous VEGF. The assay recognizes human VEGF. An antibody specific for VEGF has been pre-coated onto the microwells. The VEGF protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for VEGF is added to detect the captured VEGF protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

Vascular endothelial growth factor (VEGF), 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. The activities of VEGF are not limited to the vascular system; VEGF plays a role in normal physiological functions such as bone formation, hematopoiesis, wound healing, and development. Disruption of this gene in mice resulted in abnormal embryonic blood vessel formation. VEGF is upregulated in many known tumors and its expression is correlated with tumor stage and progression.

Sample Preparation

The serum, plasma or cell culture supernatants samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2 1:4 , is suggested according to the individual samples.

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Safety Notes

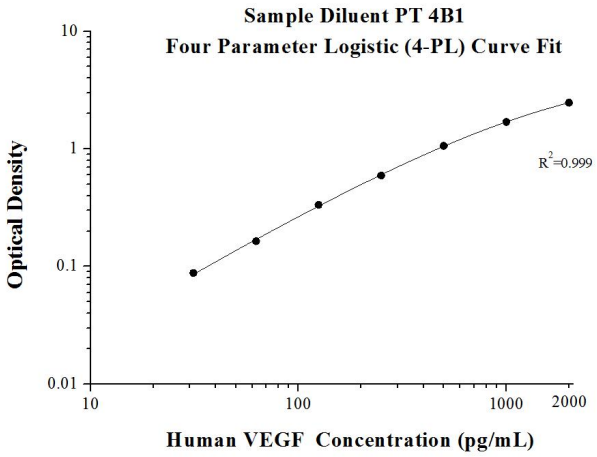
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.091 0.092	0.092	-
31.25	0.181 0.178	0.182	0.088
62.5	0.269 0.242	0.256	0.164
125	0.438 0.412	0.425	0.334
250	0.708 0.66	0.684	0.593
500	1.175 1.127	1.151	1.060
1000	1.805 1.767	1.786	1.695
2000	2.57 2.555	2.563	2.471

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Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	287.8	10.9	3.8
2	20	578.2	35.1	6.4
3	20	1,183.3	54.8	4.6

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	325.2	23.9	7.4
2	24	685.1	67.6	9.9
3	24	1,498.9	134.0	8.9

Recovery

The recovery of human VEGF spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:2	84	83-86
	1:4	83	82-85
Cell culture supernatants	1:2	86	83-89
	1:4	87	84-89

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Sample Values

Serum and plasma samples from healthy volunteers (human) were evaluated for VEGF in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human serum (n=16)	507.5	55.5-982.8
Human plasma (n=13)	127.3	14.3-385.4

cell culture supernatants:

Human peripheral blood mononuclear cells (PBMC) (1 x 10^6cells/mL) were cultured in DMEM supplemented with 8% fetal bonive serum, 5 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μg/mL PHA for 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human VEGF.

Condition	(pg/mL)
Unstimulated for 5d	230.3
Stimulated for 5d	861.3

PC-3 human prostate cancer cells were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 60 nM of PMA for 24 hours. Aliquots of the cell culture supernates were removed, assayed for levels of human VEGF.

Condition	(pg/mL)
Unstimulated for 24 hours	2,692
Stimulated for 24 hours	3,966

Sensitivity

The minimum detectable dose of human VEGF is 1 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	105	114
	Range (%)	103-109	102-108
1:8	Average% of Expected	98	114
	Range (%)	97-100	107-111
1:16	Average% of Expected	92	117
	Range (%)	81-101	109-121

References

1. Senger DR. et al. (1983). Science. 219: 983-5.
2. Ferrara N. et al. (1992). Endocr Rev. 13: 18-32.
3. Boocock CA. et al. (1995). J Natl Cancer Inst. 87: 506-516.
4. Sunderkotter C. et al. (1994). Int J Cancer. 55: 410-422.

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