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Antibodies | ELISA kits | Proteins

Human VEGF Sandwich ELISA Kit Datasheet

For the quantitative detection of human VEGF concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00085
Product Name	Human VEGF Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-1000 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 - 2000 pg/bottle; lyophilized*	2 bottles	
Detection Antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 3-ef - 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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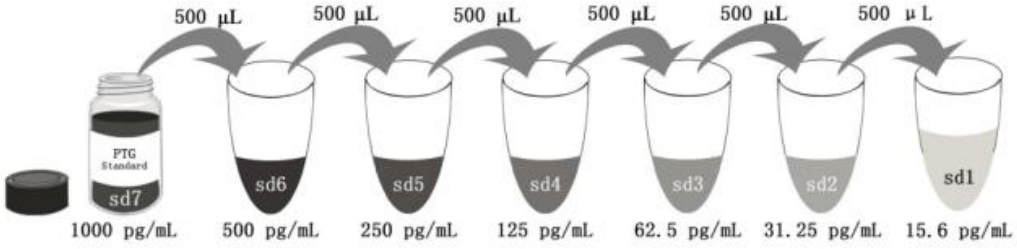
NB: Do not use the kit after the expiration date.
Sample Diluent PT 3-ef is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2 mL** Sample Diluent PT 3-ef in protein standard. This reconstitution gives a stock solution of 1000 pg/mL.



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 3-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00085 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 of biotinylated specific for VEGF is added to detect the captured VEGF protein. For signal development, Sterptravidin-HRP 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 or plasma 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

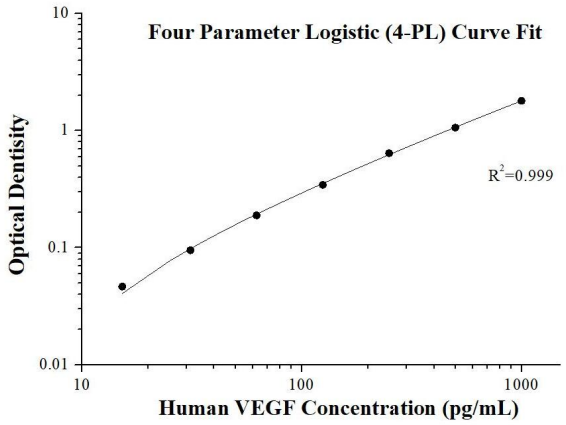
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.030 0.023	0.027	-
15.6	0.071 0.075	0.073	0.047
31.25	0.115 0.128	0.122	0.095
62.5	0.208 0.223	0.216	0.189
125	0.353 0.388	0.371	0.344
250	0.657 0.680	0.669	0.642
500	1.045 1.125	1.085	1.059
1000	1.819 1.833	1.826	1.800

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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	874.8	55.2	6.3
2	20	246.1	14.2	5.8
3	20	21.9	1.2	5.3

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	963.9	69.1	7.2
2	24	236.7	14.3	6.0
3	24	24.6	1.9	7.6

Recovery

The recovery of 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 plasma	1:2	90	81-113
	1:4	103	83-115
Cell culture supernatants	1:2	108	98-115
	1:4	108	96-118

Sample Values

Thirty-two serum and plasma samples from healthy volunteers were evaluated for human VEGF in this assay. All samples measured between 16 pg/mL and 166 pg/mL. No medical histories were available for the donors used in this study.

THP-1 cells (3 x 10^6 cells/mL) were cultured in RPMI with 10% fetal bovine serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 50 ug/mL LPS for 1, 3 and 5 days. Aliquots of the cell culture supernatants were removed and assayed for levels of natural VEGF.

Condition	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
Unstimulated	261	2,078	3,466
Stimulated	270	3,060	3,916

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Sensitivity

The minimum detectable dose of human VEGF is 6.5 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of VEGF in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Human plasma	Cell culture supernatants
1:2	Average% of Expected	88	102
	Range (%)	83-105	98-112
1:4	Average% of Expected	97	106
	Range (%)	82-111	92-110
1:8	Average% of Expected	99	108
	Range (%)	90-108	104-109
1:16	Average% of Expected	102	109
	Range (%)	86-118	107-116

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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