

Product datasheet

Human VEGF ELISA Kit ab222510

SimpleStep ELISA[®]

[3 References](#) [14 Images](#)

Overview

Product name Human VEGF ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
Supernatant	5			5.4%

Inter-assay

Sample	n	Mean	SD	CV%
Supernatant	3			5.5%

Sample type

Cell culture supernatant, Saliva, Milk, Urine, Serum, Cell culture extracts, Hep Plasma, EDTA Plasma, Cit plasma

Assay type

Sandwich (quantitative)

Sensitivity

2.7 pg/ml

Range

12.5 pg/ml - 800 pg/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Saliva	109	105% - 118%
Milk	95	91% - 98%
Urine	95	91% - 102%
Serum	109	98% - 116%
Cell culture extracts	89	80% - 94%

Sample type	Average %	Range
Cell culture media	86	81% - 89%
Hep Plasma	89	84% - 92%
EDTA Plasma	98	95% - 99%
Cit plasma	101	95% - 107%

Assay time

1h 30m

Assay duration

One step assay

Species reactivity

Reacts with: Human

Does not react with: Cow

Product overview

Human VEGF ELISA Kit (ab222510) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of VEGF protein in edta plasma, hep plasma, saliva, serum, urine, cell culture extracts, milk, cell culture supernatant, and cit plasma. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human VEGF with 2.7 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate ([ab203359](#)) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

INTERFERENCE

Serial dilutions of recombinant human VEGFR1, VEGFR2, and VEGFR3 were prepared starting at 4 ng/mL and tested for interference. No interference was observed.

SPECIES REACTIVITY

This kit recognizes human VEGF protein.

Notes

VEGF is a secreted growth factor of PDGF family active in angiogenesis, vasculogenesis and endothelial cell growth both in fetus and adult. Alternative splicing produces many isoforms

including major isoforms VEGF121, VEGF165 and VEGF189 in human. VEGF expression is induced by hypoxia. It is regulated by growth factors, cytokines, gonadotropins, nitric oxide, hypoglycemia and oncogenic mutations. VEGF induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis and induces permeabilization of blood vessels. VEGF dimers bind to the FLT1/VEGFR1 and KDR/VEGFR2 receptors, induce their homodimerization and autophosphorylation. VEGF165 and VEGF145 interact with NRP1/Neuropilin.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.

It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform Pre-coated microplate (12 x 8 well strips)

Properties

Storage instructions Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Human VEGF Capture Antibody	1 x 600µl
10X Human VEGF Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent 4BI	1 x 6ml
Human VEGF Lyophilized Recombinant Protein	1 x 2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

Function Growth factor active in angiogenesis, vasculogenesis and endothelial cell growth. Induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis and induces permeabilization of blood vessels. Binds to the FLT1/VEGFR1 and KDR/VEGFR2 receptors, heparan sulfate and heparin. NRP1/Neuropilin-1 binds isoforms VEGF-165 and VEGF-145. Isoform VEGF165B binds to KDR but does not activate downstream signaling pathways, does not activate angiogenesis and inhibits tumor growth.

Tissue specificity Isoform VEGF189, isoform VEGF165 and isoform VEGF121 are widely expressed. Isoform VEGF206 and isoform VEGF145 are not widely expressed.

Involvement in disease

Defects in VEGFA are a cause of susceptibility to microvascular complications of diabetes type 1 (MVCD1) [MIM:603933]. These are pathological conditions that develop in numerous tissues and organs as a consequence of diabetes mellitus. They include diabetic retinopathy, diabetic nephropathy leading to end-stage renal disease, and diabetic neuropathy. Diabetic retinopathy remains the major cause of new-onset blindness among diabetic adults. It is characterized by vascular permeability and increased tissue ischemia and angiogenesis.

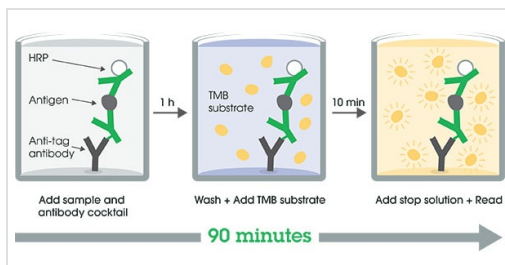
Sequence similarities

Belongs to the PDGF/VEGF growth factor family.

Cellular localization

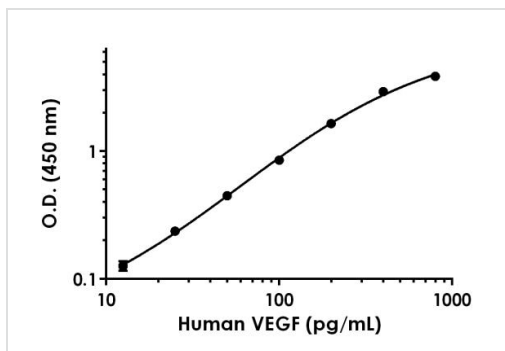
Secreted. VEGF121 is acidic and freely secreted. VEGF165 is more basic, has heparin-binding properties and, although a significant proportion remains cell-associated, most is freely secreted. VEGF189 is very basic, it is cell-associated after secretion and is bound avidly by heparin and the extracellular matrix, although it may be released as a soluble form by heparin, heparinase or plasmin.

Images



Other - Human VEGF ELISA Kit (ab222510)

SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



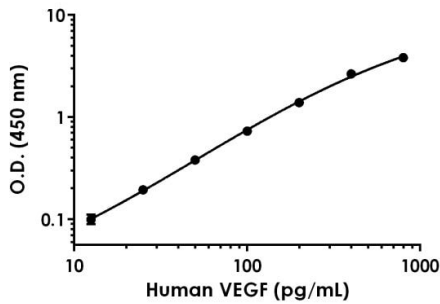
Example of human VEGF standard curve in Sample Diluent NS + 1X Enhancer.

The VEGF standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean \pm SD) are graphed.

Standard Curve Measurements			
Concentration (pg/ml)	O.D 450 nm		Mean O.D
	1	2	
0	0.104	0.100	0.102
12.5	0.236	0.221	0.229
25	0.345	0.332	0.338
50	0.558	0.540	0.549
100	0.962	0.940	0.951
200	1.740	1.750	1.745
400	3.068	2.987	3.027
800	3.959	3.954	3.957

Example of human VEGF standard curve in Sample Diluent NS + 1X Enhancer.

The VEGF standard curve was prepared as described. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



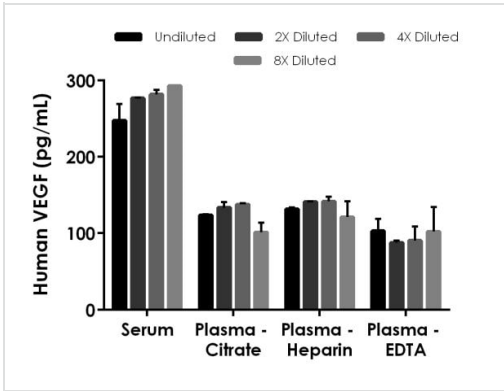
Example of human VEGF standard curve in 1X Cell Extraction Buffer PTR.

The VEGF standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Standard Curve Measurements			
Concentration (pg/ml)	O.D 450 nm		Mean O.D
	1	2	
0	0.101	0.099	0.100
12.5	0.207	0.192	0.199
25	0.304	0.284	0.294
50	0.495	0.467	0.481
100	0.853	0.805	0.829
200	1.547	1.425	1.486
400	2.879	2.634	2.757
800	3.962	3.895	3.928

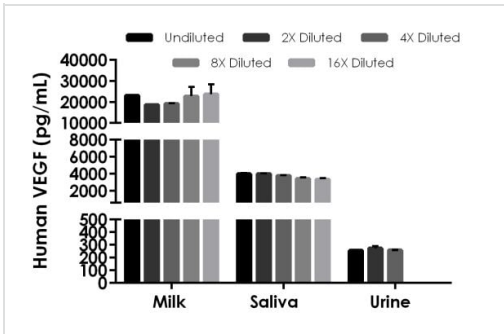
Example of human VEGF standard curve in 1X Cell Extraction Buffer PTR.

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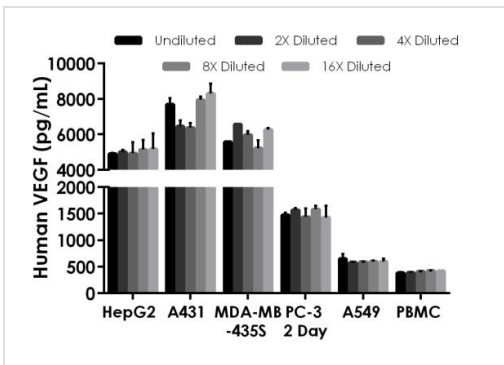
Interpolated concentrations of native VEGF in human serum and plasma samples.

The concentrations of VEGF were measured in duplicates, interpolated from the VEGF standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 50%, plasma (citrate) 50%, plasma (heparin) 50% and plasma (EDTA) 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean VEGF concentration was determined to be 268.9 pg/mL in neat serum, 123.9 pg/mL in neat plasma (citrate), 133.8 pg/mL in neat plasma (heparin), and 95.76 pg/mL in neat plasma (EDTA).



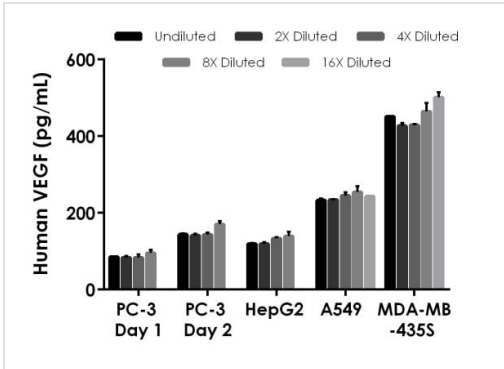
Interpolated concentrations of native VEGF in human breast milk (de-fatted), saliva, and urine samples.

The concentrations of VEGF were measured in duplicates, interpolated from the VEGF standard curves and corrected for sample dilution. Undiluted samples are as follows: breast milk 2%, saliva 12.5%, and urine 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean VEGF concentration was determined to be 21487 pg/mL in neat human breast milk (de-fatted), 3712 pg/mL in neat human saliva, and 262.0 pg/mL in neat human urine.



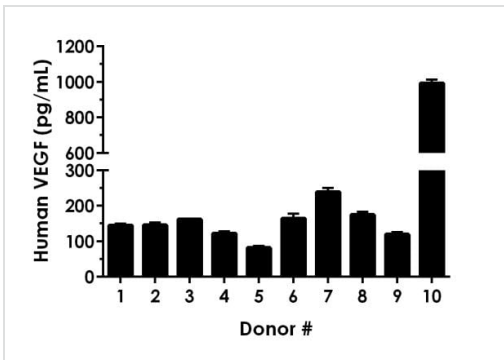
Interpolated concentrations of native VEGF in cell culture supernatant samples.

The concentrations of VEGF were measured in duplicates, interpolated from the VEGF standard curves and corrected for sample dilution. Undiluted samples are as follows: HepG2 5%, A431 5%, MDA-MB-435S 12.5%, PC-3 20%, A549 50%, and PBMC 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean VEGF concentration was determined to be 5027 pg/mL in neat HepG2 supernatant, 7356 pg/mL in neat A431 supernatant (4 Day), 5918 pg/mL in neat MDA-MB-435S supernatant, 1495 pg/mL in neat PC-3 (2 Day), 603.4 pg/mL in neat A549 supernatant, and 405.3 pg/mL in neat PBMC supernatant (PHA-M, 5 Day).



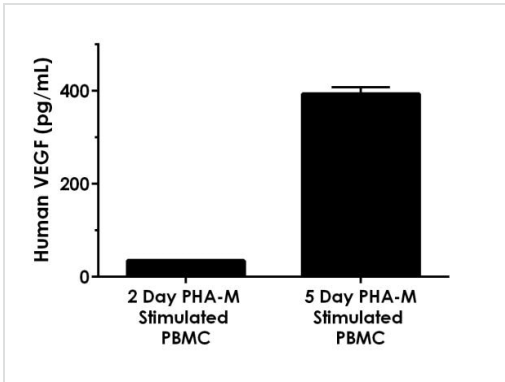
Interpolated concentrations of native VEGF.

Interpolated concentrations of native VEGF in PC-3 cell extract (1 Day), PC-3 cell extract (2 Day), HepG2 cell extract, A549 cell extract, and MDA-MB-435S cell extract samples based on a 300 µg/mL extract load. The concentrations of VEGF were measured in duplicate and interpolated from the VEGF standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean VEGF concentration was determined to be 86.65 pg/mL in PC-3 cell extract (1 Day), 149.6 pg/mL in PC-3 cell extract (2 Day), 127.7 pg/mL in HepG2 cell extract, 241.7 pg/mL in A549 cell extract, and 454.5 pg/mL in MDA-MB-435S cell extract.



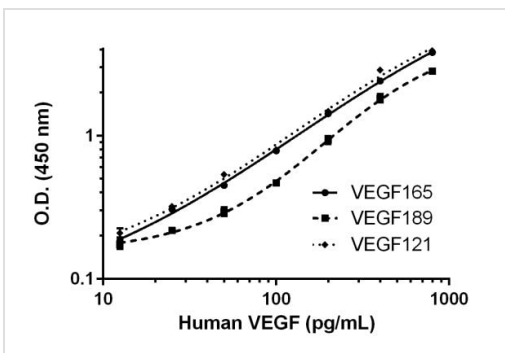
Serum from ten individual healthy human female donors was measured in duplicate.

Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean VEGF concentration was determined to be 235.0 pg/mL with a range of 81.10 – 993.3 pg/mL.



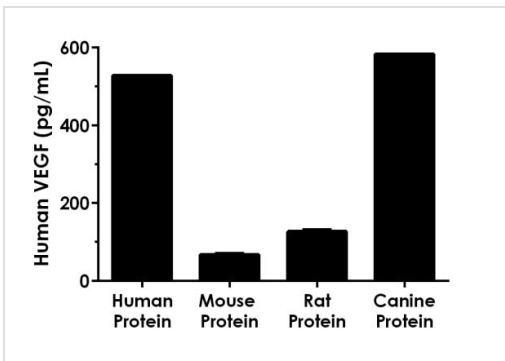
Comparison of VEGF in PHA-M stimulated and unstimulated human PBMC cell culture supernatants stimulated for different durations.

Human PBMC (seeded at $10 \times 10^6/\text{mL}$) were cultured for 2 or 5 days in the presence or absence of 1.5% PHA-M. The concentrations of VEGF were measured in three different dilutions of the supernatant samples in duplicates and interpolated from the VEGF standard curve. The interpolated values are plotted (mean \pm SD, $n=3$). The mean VEGF concentration was determined to be 34.76 pg/mL in 2 Day PHA-M stimulated PBMC cell culture supernatant, 393.7 pg/mL in 5 Day PHA-M stimulated PBMC cell culture supernatant, and undetectable in unstimulated PBMC cell culture supernatant at both 2 and 5 days (not shown).



Serial dilutions of recombinant human VEGF189 and VEGF121.

Serial dilutions of recombinant human VEGF189 and VEGF121 were prepared and assayed in parallel with recombinant VEGF165.



Other species reactivity was determined by measuring a 500 pg/mL protein load of various species recombinant VEGF proteins.

Protein concentrations were interpolated from the human standard curve, and graphing the interpolated concentrations (mean \pm SD, $n=2$).

Sample Diluent Buffer	n=	Minimal Detectable Dose
1X Cell Extraction Buffer PTR	10	2.9 pg/mL
Sample Diluent NS + 1X Enhancer	14	2.7 pg/mL

Assay sensitivity.

The MDD was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

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