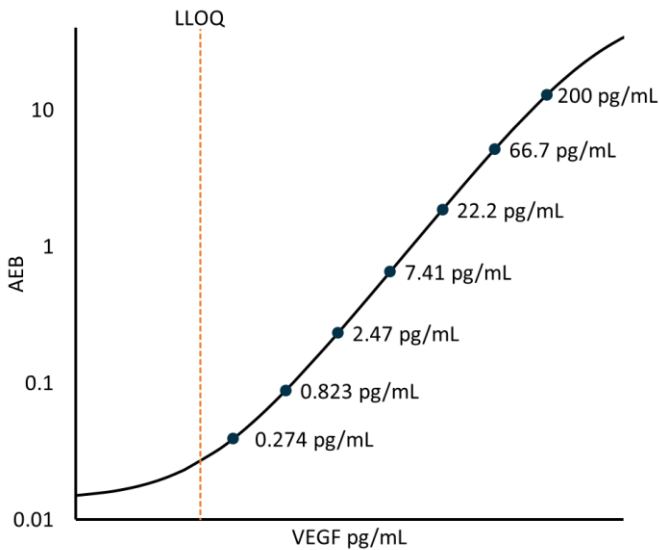


Description

Vascular endothelial growth factor (VEGF) is a 27KDa signaling protein produced by cells that stimulates vasculogenesis and angiogenesis. The VEGF family includes VEGF-A (VEGF), VEGF-B, PlGF, VEGF-C and VEGF-D. VEGF is a heparin binding protein and exists as a disulfide linked homo-dimer. It has at least 6 isoforms produced by alternative splicing. The Simoa Human VEGF assay detects the 165 amino acid form of the factor (VEGF-165), which is the most common form in tissues. VEGF induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis, and induces permeabilization of blood vessels. Though VEGF acts primarily on endothelial cells, it also binds to receptors on HSCs, monocytes, osteoblasts and neuronal cells. VEGF is also involved in vasodilation through induction of endothelial nitric oxide synthase and the subsequent increase in nitric oxide production. Serum concentration of VEGF is high in bronchial asthma and diabetes mellitus. When VEGF is over-expressed, it can contribute to disease; cancers that can express VEGF are able to grow and metastasize.

Calibration Curve: Four-parameter curve fit parameters are depicted.



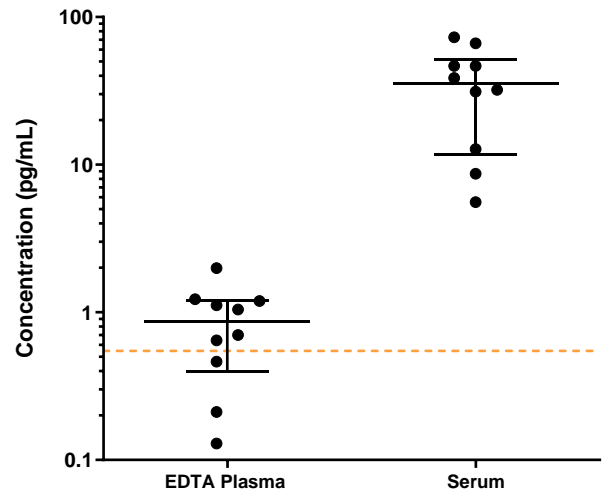
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 1 reagent lot across 4 instruments (5 runs total).

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 1 reagent lot across 4 instruments (5 runs total).

LLOQ	0.137 pg/mL pooled CV 17.9% mean recovery 97.4%
LOD	0.041 pg/mL range 0.027–0.091 pg/mL
Dynamic range (serum and plasma)	0–800 pg/mL
Diluted Sample volume*	100 µL per measurement
Tests per kit	192

*See Kit Instruction for details

Endogenous Sample Reading: Healthy donor matched EDTA plasma (n=10) and serum (n=10) were measured. Error bars depict median with interquartile range.



Sample Type	Median VEGF pg/mL	% Above LOD
Serum	141.5	100%
EDTA Plasma	3.49	100%

Precision: Representative precision was estimated with repeated assay of serum and plasma panels using four instruments and one reagent lot. Within-run and between-run CVs are depicted in the following table. Within-run CVs reflect average CVs across 5 experiments of 3 replicates each.

Sample	Mean (pg/mL)	Within run CV	Between run CV
Serum Panel 1	6.88	3.0%	5.6%
Serum Panel 2	3.51	5.9%	3.1%
Plasma Panel 3	12.4	4.2%	4.5%

Spike and Recovery (Recombinant): Recombinant VEGF spiked into 9 serum and 8 plasma samples at 400 pg/mL.

Spike and Recovery (Native): Native VEGF spiked into 3 serum and 3 plasma samples.

Dilution Linearity (Serum): 2 serum samples diluted serially from MRD (4x) to 128x with Sample Diluent.

Dilution Linearity (Plasma): 2 plasma samples diluted serially from MRD (4x) to 128x with Sample Diluent.

Spike and Recovery (Recombinant)	Mean = 110% Plasma Range: 123–150% Serum Range: 45–136%
Spike and Recovery (Native)	Mean = 103% Range: 85–120%
Dilution Linearity (Serum, 128x)	Mean = 107% Range: 93.9–124%
Dilution Linearity (Plasma, 128x)	Mean = 104% Range: 89.9–127%

Specificity: Assay signal neutralization by excess anti-VEGF antibody pre-incubated with sample.

Cross Reactivity to VEGF D: Detected concentration from 200 pg/mL added VEGF D.

Cross Reactivity to VEGF Isoforms: Isoforms 121, 145, and 189 assayed at 100 and 200 pg/mL

Specificity	98%
Cross Reactivity to VEGF D	0.01%
Cross Reactivity to VEGF Isoforms	18–48%