U-PLEX ®

Human VEGF-D

Product Options	Catalog Number	Description
Multiplex	K151AEM, K251AEM	U-PLEX Immuno-Oncology Group 1 (human)
Singleplex	K151C8K-1/-2/-4	U-PLEX Human VEGF-D Assay with SECTOR™ plates
	K151C8K-21/-22/-24	U-PLEX Human VEGF-D Assay with QuickPlex Ultra™ plates
	K251C8K-2/-4	U-PLEX Human VEGF-D Assay with 384-well plates
Antibody Set	B22C8-2/-3	U-PLEX Human VEGF-D Antibody Set
Protocol	U-PLEX Product Inserts are available at www.mesoscale.com	

The MESO SCALE DISCOVERY® U-PLEX platform was designed to provide ultimate flexibility for detection of biomarkers in a wide variety of sample types. This datasheet provides the representative performance of the U-PLEX® Human VEGF-D Assay tested on U-PLEX 96-well SECTOR plates run as a multiplex. The data do not represent the product specifications. Under your experimental conditions, the assay may perform differently from the representative data. U-PLEX assays are offered in either singleplex or multiplex; both are available on 96- or 384-well plates. See a U-PLEX product insert for instrument compatibility.

Representative Calibration Curve and Sensitivity
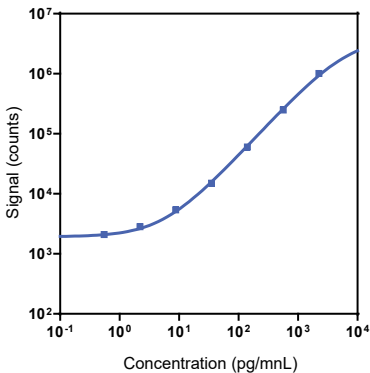
Assay	Median LLOD (pg/mL)	LLOD Range (pg/mL)
VEGF-D	0.30	0.22-0.76

The Calibrator curve was fitted with a 4-parameter logistic model with a $1/Y^2$ weighting. The lower limit of detection (LLOD) is a calculated concentration corresponding to 2.5 standard deviations above the background (zero Calibrator).

Precision

Control	Average Conc. (pg/mL)	Average Intra-run Conc. (%CV)	Inter-run Conc. (%CV)
High	280	3.8	6.8
Mid	51	5.1	13.7
Low	9.6	7.0	16.6

Controls were made by spiking Calibrator into assay diluent at 3 levels within the quantitative range of the assay. Average intra-run concentration %CV is the average %CV of the control replicates within an individual run. Inter-run concentration %CV is the variability of controls across multiple runs.

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MSD® U-PLEX Human VEGF-D

Tested Samples

Sample Type	Serum (N=10)	EDTA Plasma (N=10)	Normal Lysate (N=5)	Tumor Lysate (N=5)
Median (pg/mL)	2,630	2,720	11	2.5
Range (pg/mL)	510-5,250	883-3,840	0.60-38	ND-4.6
% Detected	100	100	100	80

Normal serum and plasma samples were diluted 4-fold prior to the assay. Lysates were tested at a protein concentration of 0.5 mg/mL. ND = non-detectable (<LLOD)

Dilution Linearity

Serum			EDTA Plasma		
Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range
2	95	91 - 98	2	95	90 - 98
8	101	99 - 103	8	101	98 - 103
16	99	96 - 101	16	97	90 - 103

Normal human serum and EDTA plasma were spiked with Calibrator and tested at different dilutions. Percent recovery at each dilution level was normalized to the dilution-adjusted, 4-fold concentration. Samples may benefit from additional dilution with assay diluent to reduce matrix effects.

% Recovery = (measured concentration / expected concentration) x 100

Spike Recovery

	Serum		EDTA Plasma	
Spike Level	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
High	102	100 - 105	102	98 - 105
Mid	102	98 - 106	104	101 - 107
Low	103	96 - 109	102	98 - 105

Normal serum and plasma were spiked with Calibrator at 3 levels. Spiked samples were diluted 4-fold to determine the expected concentration of the analyte. Samples may benefit from additional dilution with assay diluent to reduce matrix effects.

% Recovery = (measured concentration / expected concentration) x 100

Specificity

To assess specificity, the VEGF-D Antibody Set was tested individually against a larger panel of analytes for nonspecific binding: APRIL/TNFSF13, BAFF-R/TNFRSF13C, BCMA/TNFRSF17, CD20, CD27, CD276/B7-H3, CD28, CD40L (soluble), CTACK, CTLA-4, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, EPO, E-Selectin, FGF (basic), FLT3L, Fractalkine, G-CSF, Galectin-9, GITR/TNFRSF18, GITRL/TNFSF18, GM-CSF, gp130 (soluble), Granzyme A, Granzyme B, GRO-α, HAVCR2/TIM-3, HVEM/TNFRSF14, I-309, ICOS, ICOSL/B7-H2, IFN- α2a, IFN-β, IFN-γ, IL-1α, IL-1β, IL-1RA, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17A/F, IL-17C, IL-17D, IL-17E/IL 25, IL-17F, IL-18, IL-2, IL-21, IL-22, IL-23, IL-27, IL-29/IFN-λ1, IL-2Rα, IL-3, IL-31, IL-33, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, I-TAC, LAG-3, LIGHT/TNFSF14, MCP-1, MCP-2, MCP-4, M-CSF, MDC, MIF, MIG, MIP-1α, MIP-5, MMP-1, MMP-2, MMP-7, MMP-9, Nectin-4, OX40/TNFRSF4, PD1, PD-L1, PD-L2, Pentraxin 3, Perforin, PlGF, P-Selectin, RAGE (soluble), RANKL/TNFSF11, RANTES, S100A12, SDF-1α, TARC, Tie-2, TIGIT, TLR-1, TNF-RI, TNF-RII, TNF-α, TNF-β, TPO, TRAIL, TSLP, VEGF-A, VEGF-D, VEGFR-1/Flt-1 and YKL-40. Nonspecific binding was less than 2.0%.

% Nonspecificity = (nonspecific signal / specific signal) x 100

Diluent Compatibility

Diluents 58 and 3 are provided with this assay. MSD offers a range of assay and antibody diluents for separate purchase. Depending on your assay needs, other diluents may be tested.

Assay Components

Calibrator: VEGF-D is included in Calibrator 21. The human VEGF-D Calibrator is VEGF-D (93–201) expressed in an insect cell line.
Antibodies: The U-PLEX Human VEGF-D Assay uses a mouse monoclonal antibody for capture and a mouse monoclonal antibody for detection.
Assay generation: B

Note: This datasheet contains representative assay performance data. In custom multiplex formats, the assay may perform differently from the representative data shown.