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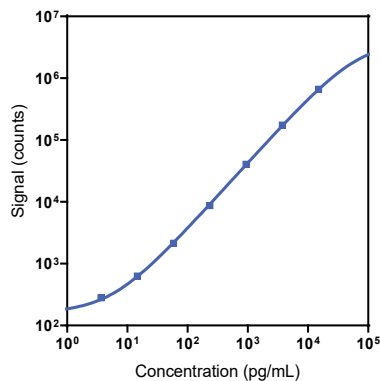
Company Address

Meso Scale Discovery, a
 division of Meso Scale
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 , LLC.
 1601 Research Boulevard
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Product Options	Catalog Number	Description
Multiplex	K151AEM, K251AEM	U-PLEX Immuno-Oncology Group 1 (human)
Singleplex	K151AHHK-1/-2/-4	U-PLEX Human VEGFR-1/Flt-1 Assay with SECTOR™ plates
	K151AHHK-21/-22/-24	U-PLEX Human VEGFR-1/Flt-1 Assay with QuickPlex Ultra™ plates
	K251AHHK-2/-4	U-PLEX Human VEGFR-1/Flt-1 Assay with 384-well plates
Antibody Set	B21AHH-2/-3	U-PLEX Human VEGFR-1/Flt-1 Antibody Set
Protocol	U-PLEX Product Inserts are available at www.mesoscale.com .	

The U-PLEX® platform was designed to provide ultimate flexibility for the detection of biomarkers in a wide variety of sample types. This datasheet provides the representative performance of the U-PLEX Human VEGFR-1/Flt-1 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)
VEGFR-1/Flt-1	2.69	1.63–4.02

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	1,850	2.9	9.3
Mid	732	2.2	7.2
Low	229	2.2	8.9

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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 Not for use in diagnostic
 procedures.

MSD® U-PLEX Human VEGFR-1/Flt-1

Tested Samples

Sample Type	Serum (N = 9)	EDTA Plasma (N = 9)	Citrate Plasma (N = 9)	Normal Lysate (N = 5)	Tumor Lysate (N = 5)
Median (pg/mL)	685	351	322	1,900	2,880
Range (pg/mL)	502–908	255–991	235–466	24–7,720	703–12,300
% Detected	100	100	100	100	100

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.

Dilution Linearity

Serum			EDTA Plasma		
Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range
2	105	97–109	2	101	93–107
8	100	97–102	8	103	100–106
16	102	98–109	16	107	102–110

Samples were spiked with calibrator and serially diluted. Percent recovery at each dilution was normalized to the dilution-adjusted 4 (or 100)-fold concentration. Samples may benefit from additional dilution with assay diluent to reduce matrix effects.

$$\% \text{ Recovery} = (\text{measured concentration} / \text{expected concentration}) \times 100$$

Spike Recovery

Spike Level	Serum		EDTA Plasma	
	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
High	130	114–141	107	100–120
Mid	129	113–148	110	102–118
Low	120	109–127	108	103–114

Samples were spiked with calibrator at three levels within the range of the assay.

$$\% \text{ Recovery} = (\text{measured concentration} / \text{expected concentration}) \times 100$$

Specificity

To assess specificity, the VEGFR-1/Flt-1 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, GTR/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- α 2a, IFN- β , IFN- γ , IL-1 α , IL-1 β , 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- λ 1, IL-2R α , 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-1 α , MIP-5, MMP-1, MMP-2, MMP-7, MMP-9, Nectin-4, OX40/TNFRSF4, PD1, PD-L1, PD-L2, Pentraxin 3, Perforin, PIGF, P-Selectin, RAGE (soluble), RANKL/TNFSF11, RANTES, S100A12, 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%.

The VEGFR-1/Flt-1 calibrator interacts with the PIGF calibrator (in Calibrator 21) which results in elevated VEGFR-1/Flt-1 calibrator signals. Measured concentrations of VEGFR-1/Flt-1 in samples are reduced by up to 34% when multiplexed with assays associated with Calibrator 21.

The VEGFR-1/Flt-1 calibrator interacts with the VEGF-A calibrator which results in altered VEGF-A signals. We will separate the VEGFR-1/Flt-1 assay and assays that share its calibrator (Calibrator 28) from the VEGF-A assay by providing it on a separate plate.

$$\% \text{ Nonspecificity} = (\text{nonspecific signal} / \text{specific signal}) \times 100$$

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: VEGFR-1/Flt-1 is included in Calibrator 28. The human VEGFR-1/Flt-1 Calibrator is a full-length recombinant protein expressed in an insect cell line.

Antibodies: The U-PLEX Human VEGFR-1/Flt-1 Assay uses a mouse monoclonal antibody for capture and a mouse monoclonal antibody for detection.

Assay generation: A

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

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