U-PLEX®

Human HVEM/TNFRSF14

Product Options	Catalog Number	Description
Multiplex	K151AEM, K251AEM	U-PLEX Immuno-Oncology Group 1 (human)
Singleplex	K151AGXK-1/-2/-4	U-PLEX Human HVEM/TNFRSF14 Assay with SECTOR™ plates
	K151AGXK-21/-22/-24	U-PLEX Human HVEM/TNFRSF14 Assay with QuickPlex® plates
	K251AGXK-2/-4	U-PLEX Human HVEM/TNFRSF14 Assay with 384-well plates
Antibody Set	B21AGX-2/-3	U-PLEX Human HVEM/TNFRSF14 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 HVEM/TNFRSF14 Assay tested on U-PLEX 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
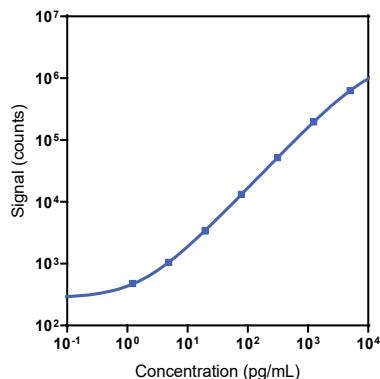
Assay	Median LLOD (pg/mL)	LLOD Range (pg/mL)
HVEM/TNFRSF14	0.53	0.34–0.88

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	104,000	2.9	5.0
Mid	29,000	1.6	4.4
Low	7,750	2.4	7.6

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 HVEM/TNFRSF14

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)	4,150	2,090	1,560	707	865
Range (pg/mL)	1,560–5,620	1,740–3,520	1,310–2,340	256–3,200	464–1,570
% 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	94	89–98	2	95	92–98
8	96	93–100	8	100	95–103
16	99	95–104	16	102	97–113

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.

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

Spike Recovery

	Serum		EDTA Plasma	
Spike Level	Average % Recovery	% Recovery Range	Average% Recovery	% Recovery Range
High	97	95–100	98	91–109
Mid	103	98–110	98	94–105
Low	102	98–103	98	92—105

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

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

Specificity

To assess specificity, the HVEM/TNFRSF14 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, 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%.

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

The HVEM/TNFRSF14 detection antibody interacted with the MMP-2 capture antibody (1.1%). We do not recommend multiplexing the HVEM/TNFRSF14 assay with the MMP-2 assay.

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: HVEM/TNFRSF14 is included in Calibrator 27. The human HVEM/TNFRSF14 Calibrator is a full-length recombinant protein.

Antibodies: The U-PLEX Human HVEM/TNFRSF14 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.