U-PLEX®

Human Nectin-4

Product Options	Catalog Number	Description
Multiplex	K151AEM, K251AEM	U-PLEX Immuno-Oncology Group 1 (human)
Singleplex	K151AHFK-1/-2/-4	U-PLEX Human Nectin-4 Assay with SECTOR™ plates
	K151AHFK-21/-22/-24	U-PLEX Human Nectin-4 Assay with QuickPlex Ultra™ plates
	K251AHFK-2/-4	U-PLEX Human Nectin-4 Assay with 384-well plates
Antibody Set	B21AHF-2/-3	U-PLEX Human Nectin-4 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 Nectin-4 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

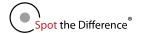| Assay	Median LLOD (pg/mL)	LLOD Range (pg/mL)
Nectin-4	1.61	1.07–2.85

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	1,390	2.6	4.9
Mid	457	1.9	5.3
Low	139	2.9	9.1

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.

Spot the Difference®

MSD® U-PLEX Human Nectin-4

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)	521	502	405	156	242
Range (pg/mL)	396–660	394–663	330–553	60–1,200	ND–7,000
% Detected	100	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	97	95–101	2	94	92–100
8	100	97–102	8	99	97–102
16	99	94–104	16	99	97–101

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) x 100

Spike Recovery

	Serum		EDTA Plasma	
Spike Level	Average % Recovery	% Recovery Range	Average% Recovery	% Recovery Range
High	102	96–107	101	92–107
Mid	107	102–112	104	89–110
Low	108	104–114	106	101–113

Samples were spiked with calibrator at three levels within the range of the assay. Percent recovery was calculated as % Recovery = (measured concentration/(spike + endogenous concentrations)) X 100.

Specificity

To assess specificity, the Nectin-4 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, 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: Nectin-4 is included in Calibrator 28. The human Nectin-4 Calibrator is a full-length recombinant protein expressed in a mouse cell line.

Antibodies: The U-PLEX Human Nectin-4 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.