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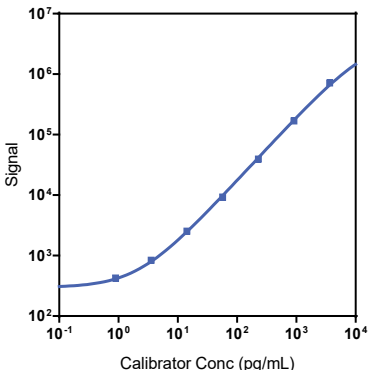
Company Address

MESO SCALE DISCOVERY[®]
 A division of
 Meso Scale Diagnostics, LLC.
 1601 Research Boulevard
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Product Options	Catalog Number	Description
Multiplex	K15067M, K25067M	U-PLEX Biomarker Group 1 (human)
	K151AEM, K251AEM	U-PLEX Immuno-Oncology Group 1 (human)
	K151ACM, K251ACM	U-PLEX Metabolic Group 1 (human)
Singleplex	K151UCK-1/-2/-4	U-PLEX Human TNF- α Assay with SECTOR [™] plates
	K151UCK-21/-22/-24	U-PLEX Human TNF- α Assay with QuickPlex [®] plates
	K251UCK-2/-4	U-PLEX Human TNF- α Assay with 384-well plates
Antibody Set	B21UC-2/-3	U-PLEX Human TNF- α Antibody Set
Protocol	U-PLEX Product Inserts are available at http://www.mesoscale.com	

The 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 TNF- α 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)
TNF- α	0.51	0.36-0.76

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	1,560	2.9	5.2
Mid	151	2.9	8.6
Low	14	3.6	13.5

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.

For Research Use Only.
 Not for use in diagnostic procedures.

MSD[®] U-PLEX Human TNF- α

Tested Samples

Sample Type	Serum (N=10)	Plasma (N=10)	Spiked Plasma (N=5)	Spiked Serum (N=5)
Median (pg/mL)	062	086	20	11
Range (pg/mL)	ND-4.2	ND-3.0	091-34	1.3-31
% Detected	50	80	100	100

Normal serum and plasma samples were diluted 2-fold prior to the assay. ND = non-detectable (<LLOD)

Dilution Linearity

Serum			EDTA Plasma		
Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range
2	109	103-113	2	107	102-111
4	112	101-117	4	109	106-115
8	118	97-156	8	104	95-114

Normal human serum and EDTA plasma were spiked with Calibrator and tested at different dilutions. Undiluted samples were tested to determine the expected concentration of the analyte. 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	115	90-125	105	100-108
Mid	107	90-116	112	108-117
Low	106	87-119	103	76-119

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

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

Specificity

To assess specificity, the TNF- α Antibody Set was tested individually against a larger panel of analytes for nonspecific binding (APRIL/TNFSF13, BAFF-R/TNFRSF13C, BCMA/TNFRSF17, BDNF, C-Peptide, CD20, CD27, CD28, CD40L (soluble), CD276/B7-H3, CTACK, CTLA-4, Desghrelin, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, EPO, E-Selectin, FGF (basic), FGF-23, FLT3L, Fractalkine, FSH, Galectin-9, G-CSF, GITRL/TNFSF18, GTR/TNFRSF18, Ghrelin (Ser3-octanoylated), gp130 (soluble), GIP (1-42), GIP (3-42), GLP-1 (7-36), GLP-1 (9-36), GM-CSF, Granzyme A, Granzyme B, GRO- α , HAVCR2/TIM-3, HVEM/TNFRSF14, ICOS, ICOS-L/B7-H2, I-309, IFN- α 2a, IFN- β , IFN- γ , IL-1 α , IL-1 β , IL-1RA, IL-2, IL-2R α , IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, 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-21, IL-22, IL-23, IL-27, IL-29/IFN- λ 1, IL-31, IL-33, Insulin, IP-10, LAG3, Leptin, LH, LIGHT/TNFSF14, MCP-1, MCP-2, MCP-4, M-CSF, MDC, MIF, MIG, MIP-1 α , MIP-1 β , MIP-5, MMP-1, MMP-2, MMP-7, Nectin-4, OX40/TNFRSF4, PD1, PD-L1, PD-L2, Pentraxin 3, Perforin, PIGF, PP, Proinsulin, proMMP-9, P-Selectin, PYY (3-36), RAGE (soluble), RANKL/TNFSF11, RANTES, S100A12, SDF-1 α , Tie-2, TIGIT, TLR1, TNF- α , TNF- β , TNF-RI, TNF-RII, TPO, TRAIL, TSLP, VEGF-A, VEGF-D, VEGFR-1/Flt-1, and YKL-40). Nonspecific binding was less than 2.0%.

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

Diluent Compatibility

Diluents 57 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: TNF- α is included in Calibrator 1. The TNF- α Calibrator is a full-length recombinant protein expressed in *E. coli*.

Antibodies: The U-PLEX Human TNF- α Assay uses a mouse monoclonal antibody for capture and a goat polyclonal 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.

