U-PLEX®

Mouse TNF-α

Product Options	Catalog Number	Description
Multiplex	K15069M, K25069M K152ACM, K252ACM	U-PLEX Biomarker Group 1 (mouse) U-PLEX Metabolic Group 1 (mouse)
Singleplex	K152UCK-1/-2/-4	U-PLEX Mouse TNF-α Assay with SECTOR™ plates
	K152UCK-21/-22/-24	U-PLEX Mouse TNF-α Assay with QuickPlex Ultra™ plates
	K252UCK-2/-4	U-PLEX Mouse TNF-α Assay with 384-well plates
Antibody Set	B22UC-2/-3	U-PLEX Mouse TNF-α 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® Mouse 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 in 96- or 384-well plates. See a U-PLEX product insert for instrument compatibility.

Representative Calibration Curve and Sensitivity

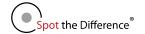| Assay	Median LLOD (pg/mL)	LLOD Range (pg/mL)
TNF-α	1.3	1.2-4.7

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 2.5X the standard deviations above the background (zero Calibrator).

Precision

Control	Average Conc. (pg/mL)	Average Intra-run Conc. (%CV)	Inter-run Conc. (%CV)
High	2,170	3.1	6.0
Mid	204	3.0	7.0
Low	22	5.5	9.6

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.

Spot the Difference®

Tested Samples

Sample Type	Serum (N=10)	Plasma (N=10)	Spiked Plasma (N=5)	Spiked Serum (N=5)
Median (pg/mL)	28	33	270	245
Range (pg/mL)	ND-36	28-36	164-395	114-486
% Detected	90	100	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	102	97-109	2	101	99-104
4	103	96-106	4	100	98-104
8	101	98-105	8	99	95-106

Normal mouse 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.

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

Spike Recovery

	Serum		EDTA Plasma	
Spike Level	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
High	89	88-91	98	94-104
Mid	85	83-89	88	85-92
Low	89	87-91	91	86-98

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.

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

Specificity

To assess specificity, the TNF-α Antibody Set was tested individually against a larger panel of analytes for nonspecific binding (6CKine/CCL21, BAFF, BCA-1/BLC, CD40, Eotaxin, EPO, GM-CSF, IFN-α, IFN-β, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17A/F, IL-17C, IL-17E/IL-25, IL-17F, IL-21, IL-22, IL-23, IL-27p28/IL-30, IL-31, IL-33, IP-10, KC/GRO, MCP-1, MCP-5/CCL12, MDC, MIP-1α, MIP-1β, MIP-2, MIP-3α, MMP-9 (total), NGAL/LCN2, RANTES, SDF-1α, TARC, TNF-RI, TNF-α, VEGF-A). Nonspecific binding was less than 0.5%.

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

Diluent Compatibility

The data included in this document have been collected with Assay Diluent 41 and Antibody Diluent 45. 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 5. The TNF-α Calibrator is a full-length recombinant protein expressed in *E. coli*.
Antibodies: The U-PLEX Mouse TNF-α Assay uses a hamster 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.