U-PLEX®

Human CTLA-4

Product Options	Catalog Number	Description
Multiplex	K151AEM, K251AEM	U-PLEX Immuno-Oncology Group 1 (human)
Singleplex	K151R7K-1/-2/-4	U-PLEX Human CTLA-4 Assay with SECTOR™ plates
	K151R7K-21/-22/-24	U-PLEX Human CTLA-4 Assay with QuickPlex® plates
	K251R7K-2/-4	U-PLEX Human CTLA-4 Assay with 384-well plates
Antibody Set	B22R7-2/-3	U-PLEX Human CTLA-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 detection of biomarkers in a wide variety of sample types. This datasheet provides the representative performance of the U-PLEX Human CTLA-4 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
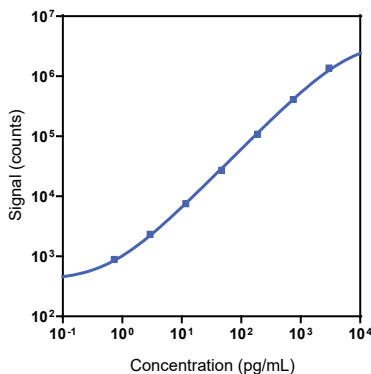
Assay	Median LLOD (pg/mL)	LLOD Range (pg/mL)
CTLA-4	0.12	0.05-0.16

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	385	3.6	6.2
Mid	67	2.7	8.7
Low	10	4.0	16.0

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 CTLA-4

Tested Samples

Sample Type	Serum (N=10)	EDTA Plasma (N=10)	Normal Lysate (N=5)	Tumor Lysate (N=5)
Median (pg/mL)	0.89	0.66	10	50
Range (pg/mL)	ND-1.0	ND-2.0	2.9-36	1.5-219
% Detected	50	50	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. ND = non-detectable (<LLOD).

Dilution Linearity

Serum			EDTA Plasma		
Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range
2	90	88 - 92	2	88	73 - 97
8	103	97 - 106	8	103	96 - 112
16	100	93 - 107	16	103	95 - 114

Normal human serum and EDTA plasma were spiked with Calibrator and tested at different dilutions. Percent recovery at each dilution level was normalized to the dilution-adjusted, 4-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	99	91 - 106	91	75 - 101
Mid	86	47 - 104	93	77 - 100
Low	120	92 - 192	89	73 - 98

Normal serum and plasma were spiked with Calibrator at 3 levels. Spiked samples were diluted 4-fold 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

The CTLA-4 Antibody Set was tested for nonspecific binding against all of the analytes in the Immuno-Oncology Group 1 and the majority of analytes in Biomarker Group 1. Any cross-reactivity greater than 2.0% is noted below. The U-PLEX Assay Designer shows all of the compatible assays.

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

Diluent Compatibility

Diluents 58 and 3 are provided when this is ordered in singleplex and multiplex assays.

Assay Components

Calibrator: CTLA-4 is included in Calibrator 20. The human CTLA-4 Calibrator is CTLA-4 (37–162) expressed in a human cell line.
Antibodies: The U-PLEX Human CTLA-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.