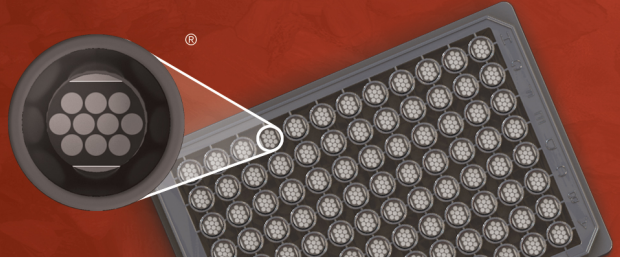
U-PLEX® Human MCP-2 Assay



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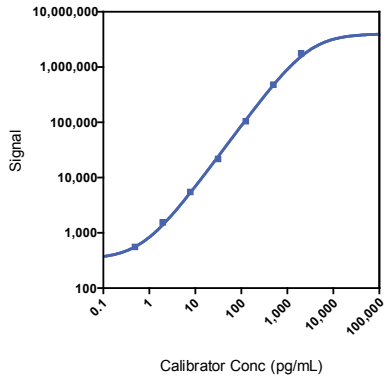
Product Options	Available as part of U-PLEX Biomarker Group 1 (hu) multiplex combination: K15067L-1/-2/-4
	Individual assay: K151XHK-1/-2/-4; Antibody Set: B21XH-2/B21XH-3
	For more ordering options, please visit www.mesoscale.com
Instrument Compatibility	SECTOR® Imager 2400, SECTOR Imager 6000, MESO® SECTOR S 600, MESO QuickPlex® SQ 120
Sample Type	Human serum, EDTA plasma, and cell culture supernatants
Assay Protocol	Refer to the U-PLEX Biomarker Group 1 (Human) product insert available at www.mesoscale.com/U-PLEX-documents

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 MCP-2 Assay tested on U-PLEX plates run as a multiplex. The data were generated during the development of the assay and do not represent the product specifications. Under your experimental conditions and with your specific multiplex, the assay may perform differently than the representative data shown. U-PLEX assays are available in multiplex format with other compatible assays. The same assay can also be used to detect a single analyte on MSD GOLD™ Small Spot Streptavidin plates.

Representative Calibration Curve and Sensitivity



Assay	Median LLOD (pg/mL)	LLOD Range (pg/mL)
MCP-2	0.11	0.11-0.17

The calibration curves used to calculate analyte concentrations were established by fitting the signals from the Calibrators using a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting. Analyte concentrations were determined from the electrochemiluminescence signals by back-fitting to the calibration curve. The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero Calibrator).

Precision

	Control	Average Conc. (pg/mL)	Average Intra-run Conc. %CV	Inter-run Conc. %CV
MCP-2	High	251	4.0	10.4
	Mid	50.4	4.7	10.1
	Low	11.3	7.0	12.8

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.

Note: This datasheet contains representative assay performance data. In custom multiplex formats, the assay may perform differently than the representative data shown.

Spike Recovery

		Serum		Plasma		Cell Culture Media	
	Spike Level	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
MCP-2	High	85	75-97	82	56-94	104	98-108
	Mid	86	78-92	92	87-96	96	93-101
	Low	92	88-101	97	96-98	93	90-95

Normal human serum, EDTA plasma, and cell culture media were spiked with Calibrator at 3 levels. Undiluted samples were tested to determine the expected concentration of the analyte. Samples may require additional dilution with assay diluent to reduce matrix effects.
% Recovery = (measured concentration / expected concentration) x 100

Tested Samples

Sample Type	Serum	Plasma	Stimulated Cell Models
Median (pg/mL)	28	21	1.6
Range (pg/mL)	20-37	18-74	ND-AS
% Detected	100	100	80

ND = non-detectable (< LLOD), AS = above standard 1

Normal serum and EDTA plasma samples were tested without dilution prior to the assay.

Dilution Linearity

	Serum			Plasma			Cell Culture Media		
	Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range
MCP-2	2	96	92-104	2	98	97-99	2	94	87-98
	4	91	87-97	4	93	89-95	4	90	79-104
	8	81	77-87	8	88	83-90	8	79	69-92

Normal human serum, EDTA plasma, and cell culture media were spiked with recombinant Calibrator and tested at different dilutions. Undiluted samples were tested to determine the expected concentration of the analyte. Samples may require additional dilution with assay diluent to reduce matrix effects.
% Recovery = (measured concentration / expected concentration) x 100

Specificity

To assess specificity, the MCP-2 Antibody Set was tested individually against a larger panel of recombinant human analytes for nonspecific binding (CTACK, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, EPO, FLT3L, Fractalkine, G-CSF, GM-CSF, GRO-α, I-309, IFN-α2a, IFN-β, IFN-γ, IL-1α, IL-1β, IL-1RA, IL-2, IL-2Rα, 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-17B, IL-17C, IL-17D, IL-17E/IL-25, IL-17F, IL-18, IL-21, IL-22, IL-23, IL-27, IL-29/IFN-λ1, IL-31, IL-33, IP-10, I-TAC, MCP-1, MCP-2, MCP-3, MCP-4, M-CSF, MDC, MIF, MIP-1α, MIP-1β, MIP-3α, MIP-3β, MIP-5, SDF-1α, TARC, TNF-α, TNF-β, TPO, TRAIL, TSLP, VEGF-A, and YKL-40). Nonspecific binding was less than 0.5%. MCP-2 detection antibody nonspecifically binds (2.8%) to Eotaxin.
% Nonspecificity = (nonspecific signal / specific signal) x 100

Diluent Compatibility

The data included in this document have been collected using Diluents 3 and 43. 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: Human MCP-2 is included in Calibrator 10 blend. The full-length recombinant human protein expressed in E.coli is used.
Antibodies: The U-PLEX Human MCP-2 Assay uses mouse monoclonal antibody for capture and rabbit polyclonal antibody for detection.
Assay generation: A