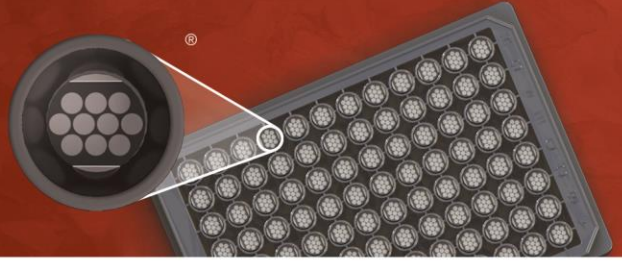
U-PLEX® Human TGF-β2 Assay



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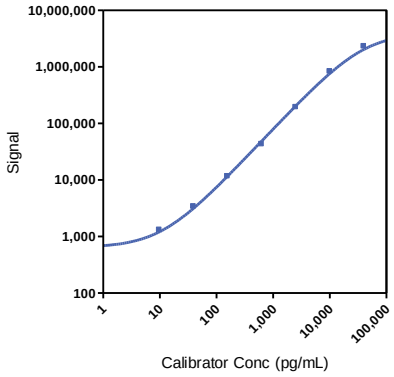
Product Options	Available as part of U-PLEX Biomarker Group 1 (hu) multiplex combination: K15067L-1/-2/-4
	Individual assay: K151XUK-1/-2/-4; Antibody Set: B20XU-2/B20XU-3
	For more ordering options, please visit www.mesoscale.com
Instrument Compatibility	SECTOR® Imager 2400, SECTOR Imager 6000, MESO® 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 TGF-β2 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)
TGF-β2	2.5	1.9-2.6

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
TGF-β2	High	5,199	4.7	10.7
	Mid	515	4.8	12.6
	Low	69	7.2	12.6

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.

Spike Recovery

		Serum		Plasma		Cell Culture Media	
	Spike Level	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
TGF-β2	High	123	109-136	70	53-82	123	109-136
	Mid	115	103-129	67	54-74	114	103-129
	Low	108	102-118	67	54-79	110	102-118

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)	ND	15	50
Range (pg/mL)	ND-31	ND-30	29-95
% Detected	40	90	100

Samples were prepared using an acidification step. Less than 2% of TGF-β2 was detected in samples not treated with acid. ND = non-detectable (< LLOD)

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
TGF-β2	2	130	123-136	2	112	99-131	2	78	74-86
	4	151	139-158	4	123	105-161	4	75	71-80
	8	163	147-171	8	129	104-172	8	68	59-76

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 TGF-β2 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, TGF-β1, TGF-β2, TGF-β3, TNF-α, TNF-β, TPO, TRAIL, TSLP, VEGF-A, and YKL-40). Nonspecific binding was less than 0.5%. TGF-β2 assay may experience small increase in signal when multiplexed with TGF-β3 assay due to interaction between capture antibodies. Samples may require additional dilution with assay diluent to reduce matrix effects.
% Nonspecificity = (nonspecific signal / specific signal) x 100

Diluent Compatibility

The data included in this document has 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 TGF-β2 is included in Calibrator 11 blend. The full-length recombinant human protein expressed in *E. coli* is used.
Antibodies: The U-PLEX Human TGF-β2 Assay uses mouse monoclonal antibody for capture and goat polyclonal antibody for detection.
Assay generation: A

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