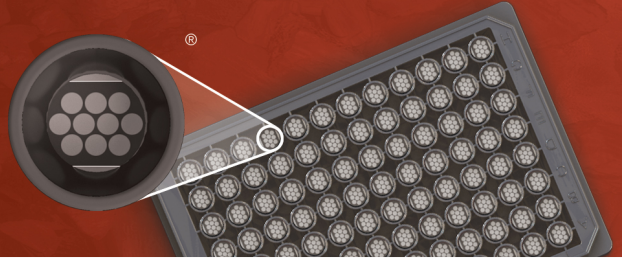
U-PLEX® Human EPO Assay



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Ordering Information

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Company Address

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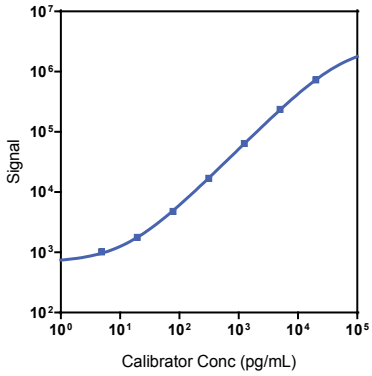
Product Options	Available in: U-PLEX Biomarker Group 1 (hu) K15067L; U-PLEX Metabolic Group 1 (hu) K151ACL
	Individual assay: K151VXK provided with Diluent 43 and Diluent 3
	Antibody Set: B21VX
Assay Protocol	U-PLEX product inserts are provided with the assays, and are available at www.mesoscale.com/U-PLEX-documents.

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 EPO 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)
EPO	1.8	1.7-2.2

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)
High	2,261	3.4	10.2
Mid	495	3.0	9.3
Low	92	4.2	15.3

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 Assays

Tested Samples

Sample Type	Serum (N=10)	Plasma (N=10)
Median (pg/mL)	154	69
Range (pg/mL)	25-251	33-477
% Detected	100	100

Normal serum and 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
2	110	107-111	2	124	108-139	2	107	102-110
4	116	110-122	4	123	107-149	4	108	104-113
8	142	133-155	8	134	115-178	8	76	71-82

Normal human serum, plasma, and cell culture media were spiked with 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.

Spike Recovery

	Serum		Plasma		Cell Culture Media	
Spike Level	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
High	90	85-96	89	62-121	83	76-90
Mid	91	86-97	98	61-158	82	72-91
Low	87	74-93	110	58-233	76	68-83

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.

Specificity

To assess specificity, the EPO Antibody Set was tested individually against a larger panel of analytes for nonspecific binding (BAFF, BDNF, β-NGF, C-Peptide, CTACK, ENA-78, Eotaxin, Eotaxin-2, Eotaxin 3, EPO, FGF-21, FGF-23, FLT3L, Fractalkine, FSH, G-CSF, Ghrelin (octanoylSer3), Desghrelin, GIP (1-42), GIP (3-42), GLP-1 (7-36), GLP-1 (9-36), Glucagon, 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, Insulin, IP-10, I-TAC, Leptin, LH, MCP-1, MCP-2, MCP-3, MCP-4, M-CSF, MDC, MIF, MIP-1α, MIP-1β, MIP-3α, MIP-3β, MIP-5, PP, Proinsulin (25-110), PYY (3-36), SDF-1α, TARC, TNF-α, TNF-β, TPO, TRAIL, TSLP, VEGF-A, YKL-40). 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 43 and Antibody Diluent 3. The assay also performed well in Assay Diluent 13 and Antibody Diluent 11. The Calibrator curve signal may differ but sample quantitation is comparable. MSD offers a range of assay and antibody diluents for separate purchase. Depending on assay needs, customers may wish to test other diluents.

Assay Components

Calibrator: Human EPO is included in Calibrator 9. The human EPO Calibrator is a full length recombinant protein expressed in chinese hamster cells.
Antibodies: The U-PLEX Human EPO Assay uses a mouse monoclonal antibody for capture and a mouse monoclonal antibody for detection.
Assay generation: B

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