

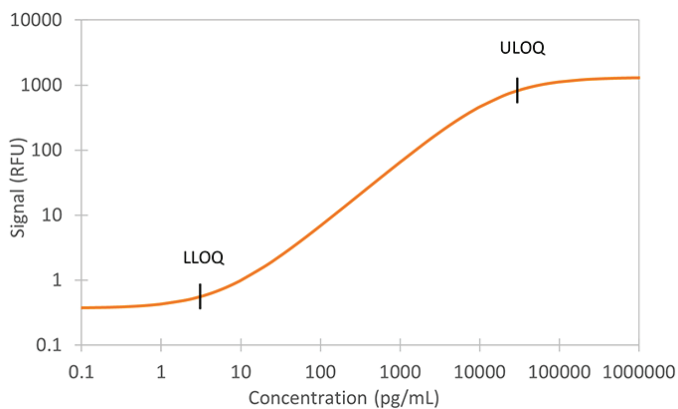
Human MPO

Simple Plex™ assay for the detection of human Myeloperoxidase (MPO) in cell culture supernatant (CCS), serum, platelet-poor plasma (EDTA/Heparin), urine, and cerebrospinal fluid (CSF).

Calibration Curve

The factory generated calibration curve shown below was compiled by averaging replicates of each calibrator from multiple runs. The 4PL curve fit shows calibrator concentration as a function of signal intensity (relative fluorescent units, RFU).

Human serum and plasma assays are quality control tested using a serum/plasma based Bio-Techne Reference Material. Curves may be adjusted to this material for lot-to-lot consistency.



Limit of Quantitation

Data shown represents typical performance results for Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) for MPO.

	Concentration (pg/mL)
LLOQ	3.14
ULOQ	30,000

Limit of Detection

The limit of detection (LOD) of human MPO is 0.598 pg/mL. The LOD was calculated by adding three standard deviations to the mean background signal determined from multiple runs.

For research use only. Not for use in diagnostic procedures.

Endogenous Levels

Endogenous levels were calculated from multiple samples. Samples were from apparently healthy volunteers. No medical histories were available for the donors in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	STD DEV (pg/mL)
Serum (n=10)	242,590	41,837 - 863,913	290,633
PP EDTA Plasma (n=10)	8726	4798 - 12,492	2237
PP Heparin plasma (n=10)	16,886	10,337 - 28,459	5642
Urine (n=6)	43,801	60 - 230,093	91,868
CSF (n=4)	2596	89 - 4723	2089

Precision

Intra-Assay Precision: Each control was tested 16 times in one assay.

Inter-Assay Precision: Replicates of each control were tested in multiple assays performed by at least three technicians using two lots of reagents.

Parameter	Intra-Assay		Inter-Assay	
	Low	High	Low	High
n	16	16	17	19
Mean (pg/mL)	178	8592	155	7619
Standard Deviation	11.2	633	14.0	714
CV (%)	6.3	7.4	9.0	9.4

Correlation

This assay has been correlated to the Quantikine™ ELISA Kit with an R² value greater than 0.9.

Recovery

Recovery at three different spiked concentrations within the range of the assay was evaluated.

Sample Type	Average%	Range%
CCS (n=2)	104	102-106

Linearity

Samples containing and/or spiked with high concentrations of human MPO were serially diluted with Sample Diluent to produce samples within the dynamic range of the assay.

Dilution	Parameter	CCS (n=7)	Serum (n=4)	Platelet-Poor	
				EDTA Plasma (n=4)	Heparin Plasma (n=4)
1:2	Avg % of Expected	94	101	99	99
	Range (%)	82-105	89-109	92-104	90-111
1:4	Avg % of Expected	93	101	103	104
	Range (%)	83-110	89-105	99-107	97-112
1:8	Avg % of Expected	89	104	100	104
	Range (%)	76-96	88-114	95-105	102-108
1:16	Avg % of Expected	106	107	99	107
	Range (%)	81-182	86-122	89-108	96-113

Dilution	Parameter	Urine (n=6)	CSF (n=3)
1:2	Avg % of Expected	89	99
	Range (%)	82-97	96-101
1:4	Avg % of Expected	97	104
	Range (%)	103	101-106
1:8	Avg % of Expected	94	96
	Range (%)	85-104	94-98
1:16	Avg % of Expected	99	91
	Range (%)	73-116	85-97

Specificity

This assay recognizes natural and recombinant human MPO. The factors listed were prepared at 50 ng/mL in Sample Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a rhMPO control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

- Integrin $\beta 2$
- MMP-12

Natural Protein:

- human Eosinophil

Sample Collection and Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

MPO is found in saliva. Take necessary precautions (e.g. mask and gloves) to prevent contamination of the kit reagents while running this assay.

Cell Culture Supernatant: Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Platelet-poor Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine: Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately, or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cerebrospinal Fluid: Freeze sample within one hour of collection to avoid aggregation. Store samples at ≤ -70 °C.

Note: Grossly hemolyzed or icteric samples are not suitable for use with this assay.

MPO is present in neutrophil granules and is released upon neutrophil exposure to activated platelets. Therefore, to measure circulating levels of MPO, platelet-free plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical Laboratory and Standards Institute (CLSI), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors released by platelet activation

Sample Preparation

CCS samples require a minimum 5-fold dilution with sample diluent. A suggested 5-fold dilution can be achieved by adding 20 μ L of sample to 80 μ L of Sample Diluent. Samples above the ULOQ require further dilution.

Serum samples require a minimum 100-fold dilution with sample diluent. A suggested 100-fold dilution can be achieved by adding 10 μ L of sample to 90 μ L of Sample Diluent (Dilution 1). Complete the 100-fold dilution by adding 10 μ L of Dilution 1 to 90 μ L of Sample Diluent.

Plasma, urine, and CSF samples require a minimum 10-fold dilution with sample diluent. A suggested 10-fold dilution can be achieved by adding 10 μ L of sample to 90 μ L of Sample Diluent. Samples above the ULOQ require further dilution.

