

Human MPO Sandwich ELISA Kit Datasheet

For the quantitative detection of human MPO concentrations in serum, plasma, cell culture supernatants, cell lysates and saliva.

General Information

Catalogue Number	KE00171
Product Name	Human MPO Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	4353
SwissProt	P05164

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1 - 30 mL/bottle. For serum, plasma and saliva	1 bottle	
Sample Diluent PT 1-sc - 30 mL/bottle. For cell culture supernatants and cell lysates	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

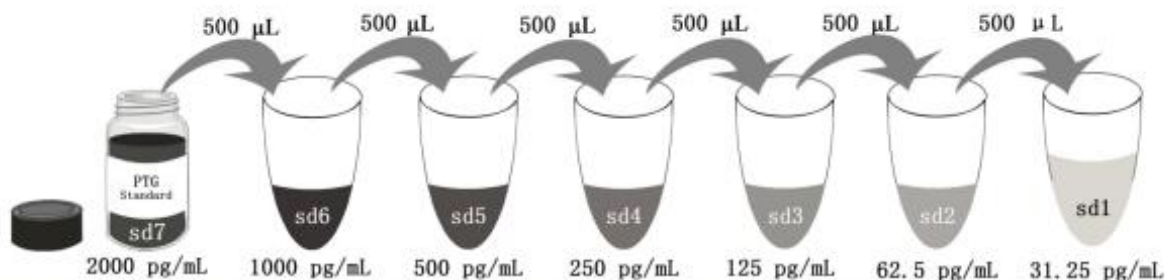
NB: Do not use the kit after the expiration date.

Sample Diluent PT 1 is for protein standard, serum, plasma and saliva.

Sample Diluent PT 1-sc is for protein standard, cell culture supernatants and cell lysates.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 2 mL Sample Diluent PT 1 or PT 1-sc in protein standard. This reconstitution gives a stock solution of 2000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1 or PT 1-sc	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00171 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The MPO ELISA kit is to be used to detect and quantify protein levels of endogenous MPO. The assay recognizes Human MPO. An antibody specific for MPO has been pre-coated onto the microwells. The MPO protein in samples is captured by the coated antibody after incubation.

Following extensive washing, another antibody of biotinylated specific for MPO is added to detect the captured MPO protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

MPO(myeloperoxidase) is a peroxidase enzyme presented in the azurophilic granules of polymorphonuclear (PMN) leukocytes and monocytes. Plasma concentration of MPO can be used as a specific marker of PMN activation. MPO catalyzes the production of hypochlorous acid (HClO) from hydrogen peroxide (H₂O₂) and chloride anion (Cl⁻, or the equivalent from a non-chlorine halide). This enzymatic system plays an important role in human defense against microorganisms. The serum/plasma MPO levels have been associated with a variety of clinical conditions including inflammatory diseases, atherosclerosis, ischaemic stroke, hypertension, heart failure, risk of cardiovascular events and so on.

Sample Preparation

Different samples may require proper dilution to fall within the range of the assay. The serum or plasma is better to be diluted 1:400 or 1:800 before assay, 1:20 or 1:40 dilution is recommended for cell culture supernatants, 1:4,000 or 1:8,000

dilution is recommended for cell lysates, 1:2,000 or 1:4,000 dilution is recommended for saliva.

Safety Notes

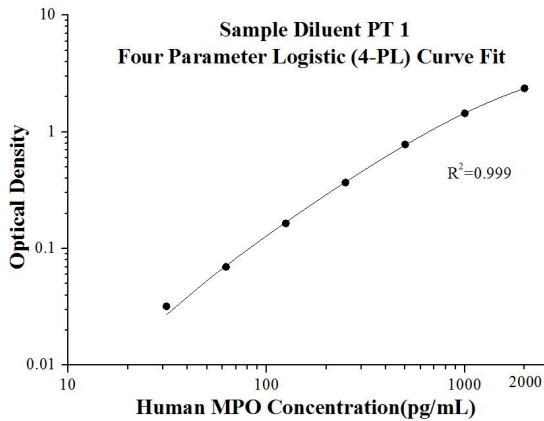
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

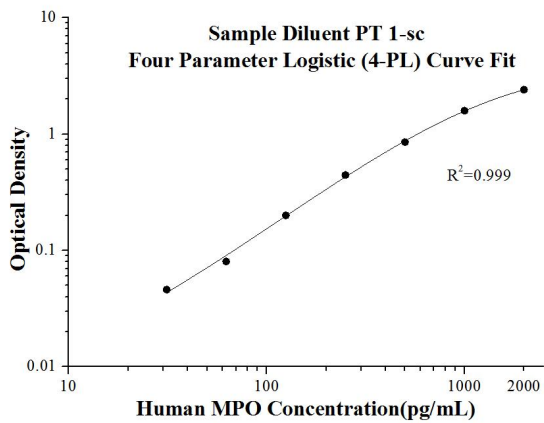
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.044 0.049	0.047	-
31.25	0.079 0.078	0.079	0.032
62.5	0.111 0.121	0.116	0.070
125	0.204 0.217	0.211	0.164
250	0.406 0.422	0.414	0.368
500	0.794 0.857	0.826	0.779
1000	1.496 1.48	1.488	1.442
2000	2.387 2.427	2.407	2.361



(pg/mL)	O.D	Average	Corrected
0	0.096 0.107	0.102	-
31.25	0.135 0.16	0.148	0.046
62.5	0.178 0.186	0.182	0.081
125	0.299 0.304	0.302	0.200
250	0.547 0.545	0.546	0.445
500	0.963 0.946	0.955	0.853
1000	1.648 1.727	1.688	1.586
2000	2.487 2.522	2.505	2.403

Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	974.4	19.5	2.0
2	20	239.6	6.8	2.8
3	20	50.9	4.0	7.8

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	961.5	36.4	3.8
2	24	242.7	12.7	5.2
3	24	53.4	4.4	8.3

Recovery

The recovery of MPO spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:1,600	111	88-126
	1:3,200	90	80-105
Cell culture supernatants	1:80	98	89-117
	1:160	95	81-125
Cell lysates	1:15,000	102	91-110
	1:30,000	108	97-125
Saliva	1:6,000	96	88-110
	1:12,000	96	87-106

Sample Values

Serum, plasma, saliva and urine samples from volunteers were evaluated for MPO in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human serum (n=16)	639	310-1,071
saliva (n=8)	1,392	131-3,611

Cell culture supernatants:

HL-60 were cultured in DMEM supplemented with 10% fetal bovine serum, 2.5 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human MPO, and measured 7.4 ng/mL.

Cell lysates -Dissect the tissue of interest and wash briefly with chilled **1X PBS** to remove any blood if necessary, cut the tissue into smaller pieces whilst keeping it on ice. Transfer the tissue to a homogenizer and add **Extraction Reagent** with protease inhibitor. In general, add 500 μ L **Extraction Reagent** for approximately every 10 mg of tissue. Homogenize thoroughly and keep the sample on ice for 30 min. Sonicate the sample and centrifuge at 10,000 x g, then transfer the supernatant to assay.

	Human MPO (ng/mL)	Total protein (mg/mL)
HL-60	1,548	6

***1X PBS** For 1000 mL

10 mM Na₂HPO₄, 1.8 mM NaH₂PO₄, 140 mM NaCl. Adjust pH to 7.4 and add ddH₂O to 1000 mL.

Sensitivity

The minimum detectable dose of Human MPO is 9.1 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

(The serum samples were initially diluted 1:200. The cell culture supernatants samples were initially diluted 1:10. The cell lysates samples were initially diluted 1:2,000. The saliva samples were initially diluted 1:1,000.)

		Human serum (Sample Diluent PT 1)	Cell culture supernatants (Sample Diluent PT 1-sc)	Cell lysates (Sample Diluent PT 1-sc)	Saliva (Sample Diluent PT 1)
1:2	Average% of Expected	100	100	100	100
	Range (%)	-	-	-	-
1:4	Average% of Expected	94	112	106	99
	Range (%)	90-100	100-128	103-108	91-109
1:8	Average% of Expected	90	115	116	96
	Range (%)	82-102	108-119	111-122	88-104
1:16	Average% of Expected	108	117	105	115
	Range (%)	96-121	108-127	77-122	111-120

References

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