Human MPO Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00171

Size: 96T

Sensitivity: 9.1 pg/mL

Range: 31.25-2000 pg/mL

Usage: For the quantitative detection of human MPO concentrations in serum, plasma, cell culture supernatant, saliva and cell lysate.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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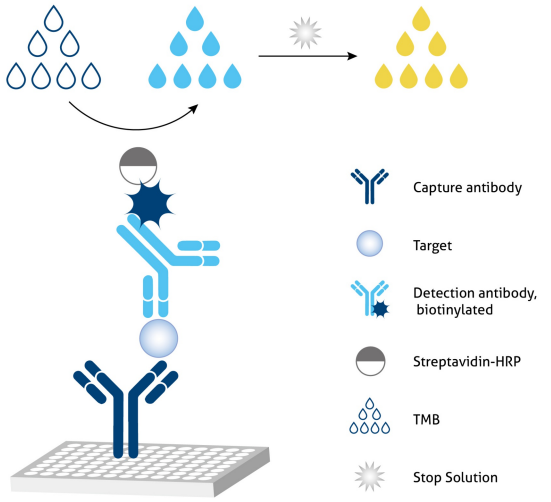
1. Background

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.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with biotin)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with biotin also binds to the analyte. Streptavidin-HRP binds to the biotin. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

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4. Kit Components and 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 (100×) - 120 µL/vial*	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 1 - 30 mL/bottle. For human serum, plasma and saliva	1 bottles	
Sample Diluent PT 1-sc - 30 mL/bottle. For cell culture supernatant and cell lysate	1 bottles	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 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	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

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6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.4 Saliva: Collect saliva samples and centrifuge for 5 minutes at 10,000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.5 Cell Lysate:

1) Collect cells and wash by centrifuging at 500 x g for 5 minutes before resuspension in pre-cooled PBS buffer. Perform this step three times.

2) Count cells and then discard the supernatant.

3) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing cell lysis.

4) Add 1 mL of Extraction reagent (containing protease inhibitor cocktail) Per 1 x 107 cells, Incubate cell suspension on ice for 30 minutes, use ultrasound to treat the samples.

5) Centrifuge cell lysate at 10,000 x g for 5 minutes at 4°C.

6) Measure the concentration of total protein in cell lysate using BCA assay. Where possible, keep samples on ice to avoid protein degradation.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody (1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

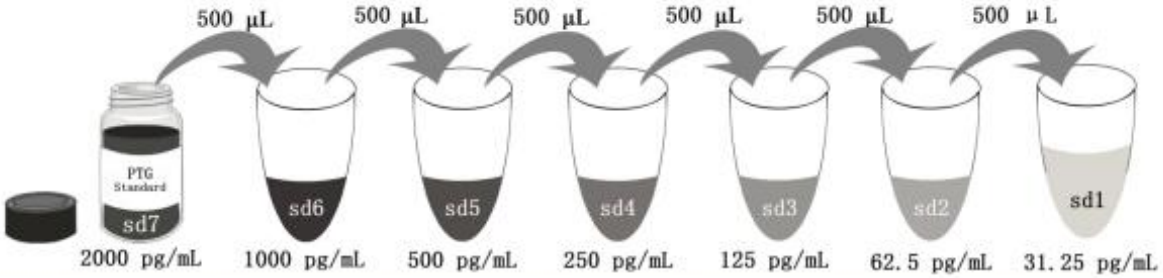
7.3 Streptavidin-HRP (1X): Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Streptavidin-HRP + 990 µL Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:400 or 1:800 is recommended for human serum and plasma; 1:20 or 1:40 is recommended for cell culture supernatant; 1:4,000 or 1:8,000 is recommended for cell lysate; 1:2,000 or 1:4,000 is recommended for saliva.

7.5 Standard Serial Dilution:

For human serum, plasma and saliva samples, add 2 mL Sample Diluent PT 1 in protein standard; For cell culture supernatant and cell lysate samples, add 2 mL Sample Diluent PT 1-sc in protein standard.



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"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and Streptavidin-HRP can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X Streptavidin-HRP solution (refer to Reagent Preparation7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C .

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

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.				

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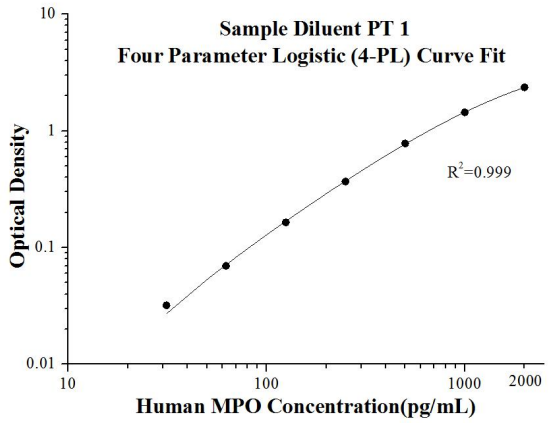
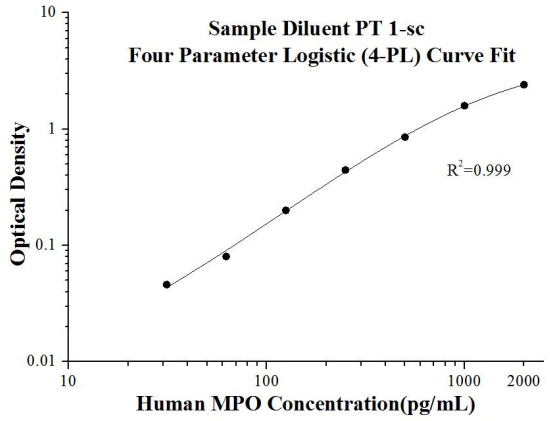
9. Validation Data

9.1 Standard curve

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

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9.2 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

9.3 Recovery

The recovery of human MPO spiked to three different levels 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 supernatant	1:80	98	89-117
	1:160	95	81-125
Cell lysate	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

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9.4 Sample values

Human serum and saliva samples from volunteers were evaluated for human 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 supernatant:

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

Cell lysate -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 µL **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 Na_2HPO4, 1.8 mM NaH_2PO4, 140 mM NaCl. Adjust pH to 7.4 and add ddH_2O to 1000 mL.

9.5 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.

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9.6 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 human serum samples were initially diluted 1:200. The cell culture supernatant samples were initially diluted 1:10. The cell lysate samples were initially diluted 1:2,000. The saliva samples were initially diluted 1:1,000.)

		Human serum (Sample Diluent PT 1)	Cell culture supernatant (Sample Diluent PT 1-sc)	Cell lysate (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

10. References

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