

PRODUCT INFORMATION AND MANUAL

Human MPO Instant ELISA

BMS2038INST

Enzyme-linked immunosorbent assay for quantitative
detection of human MPO.

For research use only.

Not for diagnostic or therapeutic procedures.

128 Tests



**human MPO
BMS2038INST**

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1 Intended Use

The human MPO Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human Myeloperoxidase. **The human MPO Instant ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

2 Summary

Myeloperoxidase (MPO) is a human enzyme in the azurophilic granules of neutrophils and in the lysosomes of monocytes. Its major role is to aid in microbial killing.

MPO is a dimeric molecule, consisting of a pair of heavy- and light-chain protomers and 2 iron atoms. The gene for MPO is located on band 17q22-23. MPO has only 1 gene; thus, the mature enzyme is synthesized from a single polypeptide product. The synthesis of MPO primarily occurs during the promyelocytic stage of myeloid development, concurrent with development of the azurophilic granules. The MPO gene encodes for a primary translational product, which is glycosylated to yield an enzymatically inactive precursor, apopro-MPO. Heme is then inserted, yielding the enzymatically active precursor, pro-MPO.

MPO is found in the azurophilic granules of neutrophilic polymorphonuclear leukocytes (PMNs) and in the lysosomes of monocytes in humans. MPO is most abundant in the granules of neutrophils. Monocytes contain only about a third of the MPO present in neutrophils.

Normal function of myeloperoxidase:

When neutrophils become activated in conjunction with phagocytosis, they undergo a process referred to as a respiratory burst. This respiratory burst causes production of superoxide, hydrogen peroxide, and other reactive oxygen derivatives, which are all toxic to microbes. During respiratory bursts, granule contents are released into the phagolysosomes and outside the cell, allowing released contents to come into contact with any microbes present. MPO catalyzes the conversion of hydrogen peroxide and chloride ions (Cl) into hypochlorous acid. Hypochlorous acid is 50 times more potent in microbial killing than hydrogen peroxide.

MPO also chlorinates phagocytosed bacteria directly; thus, the MPO–hydrogen peroxide–Cl system seems to have an important role in microbial killing. In addition to killing bacteria, the products of the MPO–hydrogen peroxide–Cl system are believed to play a role in killing fungi,

parasites, protozoa, viruses, tumor cells, natural killer (NK) cells, red cells, and platelets. The MPO–hydrogen peroxide–Cl system is also believed to be involved in terminating the respiratory burst, because individuals with MPO deficiency have prolonged respiratory bursts. It may play a role in down-regulating the inflammatory response. MPO may have a role in atherosclerosis, carcinogenesis, and degenerative neurological diseases. The role of MPO in atherosclerosis is supported by the fact that MPO deficiency may protect against cardiovascular disease.

MPO deficiency is associated with a huge number of disorders like Thrombotic diseases, Renal transplantation, Diabetes mellitus, Neuronal lipofuscinosis, several hematologic disorders and neoplasms, Obesity, Acute myeloid leukemia (AML), Chronic myeloid leukemia (CML), Polycythemia vera, Hodgkin disease, Refractory megaloblastic anemia, Aplastic anemia, Myelofibrosis with myeloid metaplasia, Myelodysplastic syndromes.

3 Principles of the Test

An anti-human MPO coating antibody is adsorbed onto microwells. Human MPO present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated anti-human MPO antibody binds to human MPO captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-human MPO.

Following incubation unbound biotin conjugated antihuman MPO and Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of soluble human MPO present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human MPO standard dilutions and human MPO sample concentration determined.

Figure 1

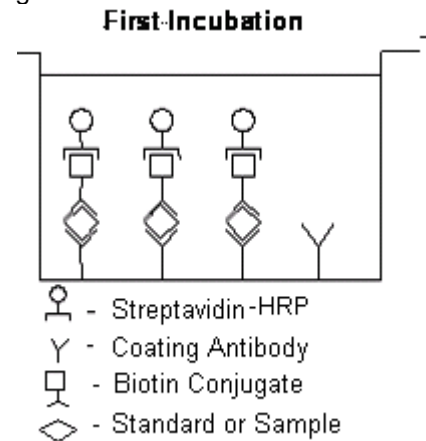


Figure 2

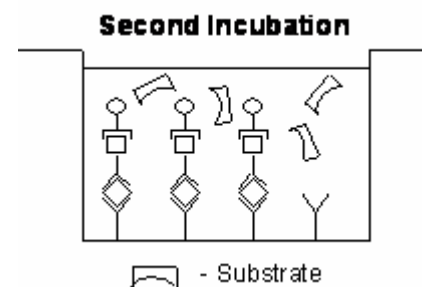
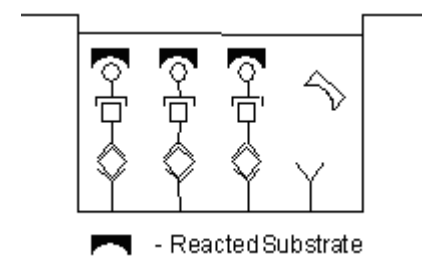


Figure 3



4 Reagents Provided

- 1 aluminium pouch with a **Microwell Plate coated** with monoclonal antibody to human MPO, **Biotin-Conjugate** (anti-human MPO monoclonal antibody), **Streptavidin-HRP** and Sample Diluent, lyophilized
- 2 aluminium pouches with a human MPO **Standard curve** (coloured)
- 1 bottle (25 ml) **Wash Buffer Concentrate 20x** (phosphate-buffered saline with 1% Tween 20)
- 1 vial (12 ml) **Sample Diluent** (Use when an external predilution of the samples is needed)
- 1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)
- 1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)
- 2 **Adhesive Films**

5 Storage Instructions

Store ELISA plate and Standard curves or whole kit at -20°C. The plate and the standard curves can also be removed, stored at -20°C, remaining kit reagents can be stored between 2° and 8°C. Expiry of the kit and reagents is stated on labels.

The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6 Specimen Collection

Cell culture supernatant, serum and plasma (EDTA, citrate, heparinized) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Pay attention to a possible “**Hook Effect**” due to high sample concentrations (see chapter 11).

Samples must be stored frozen at -20°C to avoid loss of bioactive human MPO. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7 Materials Required But Not Provided

- 5 ml and 10 ml graduated pipettes
- 5 μ l to 1000 μ l adjustable single channel micropipettes with disposable tips
- adjustable multichannel micropipettes (for volumes between 50 μ l and 500 μ l) with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

8 Precautions for Use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.

- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 Preparation of Reagents and Samples

The **buffer concentrate** should be brought to room temperature and diluted before starting the test procedure.

9.1 Wash Buffer

If crystals have formed in the **Wash Buffer Concentrate**, warm it gently until they have completely dissolved.

Pour entire contents (25 ml) of the Wash Buffer Concentrate into a clean 500 ml graduated cylinder. Bring to final volume to 500 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer is stable for 30 days.

10 Test Protocol

- **Use plate immediately after removal from -20 °C!**
 - **Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!**
 - **Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results.**
 - **Perform the washing step with at least 400 µl of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results.**
 - **Allow the washing buffer to sit in the wells for a few seconds before aspiration.**
 - **Remove covers of the standard strips carefully so that all the lyophilized pellets remain in the wells.**
- a. Prepare your samples before starting with the test procedure. Dilute serum or plasma samples 1:50 with **Sample Diluent** according to the following dilution scheme:
10 µl sample + 490 µl Sample Diluent
- b. Determine the number of Microwell Strips required to test the desired number of samples plus Microwell Strips for blanks and standards (coloured). Each sample, standard and blank should be assayed in duplicate. Remove extra Microwell Strips from holder and store in foil bag with the desiccant provided at -20 °C sealed tightly. Place Microwell Strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).
- c. Add 100 µl of **distilled water** to the **sample wells**.
- d. Add **distilled water** to all **standard and blank wells** as indicated on the label of the standard strips (A1, A2 to H1, H2).

Table 1

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (10000 pg/ml)	Standard 1 (10000 pg/ml)	Sample 1	Sample 1
B	Standard 2 (5000 pg/ml)	Standard 2 (5000 pg/ml)	Sample 2	Sample 2
C	Standard 3 (2500 pg/ml)	Standard 3 (2500 pg/ml)	Sample 3	Sample 3
D	Standard 4 (1250 pg/ml)	Standard 4 (1250 pg/ml)	Sample 4	Sample 4
E	Standard 5 (625 pg/ml)	Standard 5 (625 pg/ml)	Sample 5	Sample 5
F	Standard 6 (313 pg/ml)	Standard 6 (313 pg/ml)	Sample 6	Sample 6
G	Standard 7 (156 pg/ml)	Standard 7 (156 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

- e. Add 50 µl of each 1:50 prediluted **sample**, in duplicate, to the **designated wells** and mix the contents.
- f. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 3 hours, if available on a microplate shaker at 200 rpm.
- g. Remove adhesive film and empty wells. **Wash** the microwell strips 4 times with approximately **400 µl** Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.

After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.

- h. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.
- i. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.

It is recommended to add the Stop Solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.6 – 0.65.

- j. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

- k. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human MPO standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

11 Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human MPO concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human MPO for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human MPO concentration.
- ***Samples have been diluted 1:100, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 100).**
- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human MPO levels (Hook Effect). Such samples require further external predilution according to expected human MPO values with Sample Diluent in order to precisely quantitate the actual human MPO level.**
- It is suggested that each testing facility establishes a control sample of known human MPO concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 4. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

* N.B: There is a common dilution factor for samples due to the conjugate which must then be included in the calculation. The samples contribute 100 µl to the final volume per well. These 100 µl are composed of 50 µl of Sample Diluent plus 50 µl of the 1:50 prediluted sample. This is a 1:100 dilution.

The remaining 50 µl to give 150 µl are due to the addition of 50 µl conjugate to all wells.

50 µl Sample Diluent and 50 µl conjugate results in 100 µl reconstitution volume, addition of 50 µl 1:50 prediluted sample (50 µl + 50 µl 1:50 prediluted sample = 1:100 dilution)

Figure 4

Representative standard curve for human MPO Instant ELISA. Human MPO was diluted in serial 2-fold steps in Sample Diluent. Each symbol represents the mean of 3 parallel titrations.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

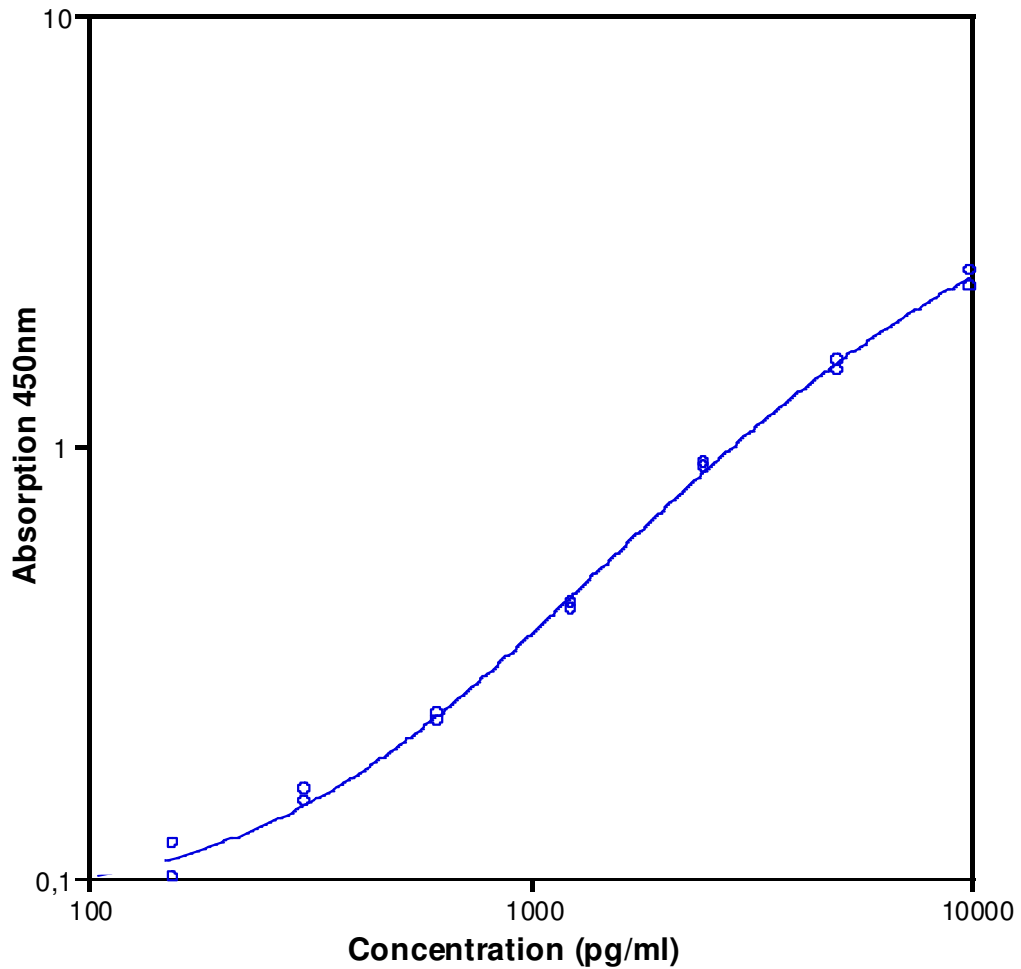


Table 2

Typical data using the human MPO INSTANT ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	human MPO Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	10000	2,35	2,45	6,1
	10000	2,56		
2	5000	1,50	1,53	3,5
	5000	1,57		
3	2500	0,89	0,90	1,7
	2500	0,91		
4	1250	0,43	0,42	0,9
	1250	0,42		
5	625	0,24	0,23	2,5
	625	0,23		
6	313	0,15	0,16	1,0
	313	0,16		
7	156	0,12	0,11	8,1
	156	0,10		
Blank	0	0,06	0,06	7,6
	0	0,05		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

12 Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human MPO defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0,026 ng/ml (mean of 6 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human MPO. 2 standard curves were run on each plate. Data below show the mean human MPO concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 6,2%.

Table 3

The mean human MPO concentration and the coefficient of variation for each sample.

Positive Sample	Experiment	human MPO Concentration (ng/ml)	Coefficient of Variation (%)
1	1	549,6	4,4
	2	422,3	5,6
	3	477,3	4,5
2	1	234,5	5,7
	2	187,8	8,8
	3	223,9	2,9
3	1	258,7	6,2
	2	221,0	8,0
	3	272,9	4,2
4	1	81,3	10,8
	2	85,7	6,0
	3	79,8	7,3
5	1	40,6	6,9
	2	30,3	4,2
	3	38,7	4,8
6	1	85,0	4,0
	2	83,1	8,3
	3	88,5	9,0

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human MPO. 2 standard curves were run on each plate. Data below show the mean human MPO concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 9,5%.

Table 4

The mean human MPO concentration and the coefficient of variation of each sample

Sample	Mean human MPO Concentration (ng/ml)	Coefficient of Variation (%)
1	483,1	13,2
2	215,4	11,4
3	250,9	10,7
4	82,3	3,7
5	36,5	15,1
6	85,5	3,2

13.3 Spike Recovery

The spike recovery was evaluated by spiking human MPO into different matrices (serum, plasma, cell culture supernatant). Recoveries were determined in 4 independent experiments with 2 replicates each (see Table 5). The unspiked matrices were used as blank in these experiments. The amount of endogenous human MPO in unspiked matrices was subtracted from the spike values.

Table 5

Matrix	Mean recovery of 4 experiments (%)
serumpool	87,0
plasmapool	86,9
cell culture supernatant	69,0

13.4 Dilution Parallelism

Samples with different levels of human MPO were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged between 74% and 108% with an overall recovery of 88% (see Table 6).

Table 6

Sample	Dilution	Mean human MPO Concentration (ng/ml)		% Recovery of Exp. Val.
		Expected Value	Observed Value	
1	1:100		161,8	
	1:200	80,9	85,8	106
	1:400	40,5	35,8	88
	1:800	20,2	19,5	96
2	1:100		80,4	
	1:200	40,2	43,4	108
	1:400	20,1	15,5	77
	1:800	10,1	8,5	85
3	1:100		80,9	
	1:200	40,4	35,6	88
	1:400	20,2	15,0	74
	1:800	10,1	7,5	75
4	1:100		194,3	
	1:200	97,2	97,2	100
	1:400	48,6	42,9	88
	1:800	24,3	18,5	76

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed 5 times, and the human MPO levels determined.

There was no significant loss of human MPO immunoreactivity detected by freezing and thawing.

13.5.2 Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C and room temperature (RT), and the human MPO level determined after 24 h.

There was no significant loss of human MPO immunoreactivity detected.

13.6 Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into positive serum.

There was no crossreactivity detected.

13.7 Expected Values

A panel of sera and plasma samples from randomly selected apparently healthy donors (males and females) was tested for human MPO.

The detected human MPO levels ranged between 1,45 and 72,67ng /ml with a mean level of 17,02 ng/ml.

The levels measured may vary with the sample collection used.

14 Bibliography

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For literature update refer to www.bendermedsystems.com

15 Ordering Information

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Cat.No. BMS2038INST human MPO INSTANT ELISA

16 Reagent Preparation Summary

16.1 Wash Buffer

Add **Wash Buffer Concentrate** 20 x (25 ml) to 475 ml distilled water

17 Test Protocol Summary

- Predilute sample 1:50 with Sample Diluent
- Place standard strips in position A1/A2 to H1/H2.
- Add 100 µl distilled water to sample wells.
- Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 50 µl 1:50 prediluted sample to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25 °C) if available on a microplate shaker at 200 rpm.
- Empty and wash microwell strips 4 times with 400 µl Wash Buffer.
- Add 100 µl of TMB Substrate Solution to all wells including blank wells.
- Incubate the microwell strips for about 10 minutes at room temperature (18° to 25 °C).
- Add 100 µl Stop Solution to all wells including blank wells.
- Blank microwell reader and measure colour intensity at 450 nm.

Note: Samples have been diluted 1:100, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 100).