Mouse Myeloperoxidase (MPO) Detection ELISA Kit

Catalog # 6051

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse myeloperoxidase (MPO)
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	6 hours
STANDARD RANGE:	0.8 - 50 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Liquid samples and biological fluids (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (3.0-6.1%)/Inter-Assay (7.2-7.5%)/Spiking Test (93-95%)
NOTES:	N/A

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INTRODUCTION

Neutrophils are crucial immune cells that defend the body against bacterial and viral infection through phagocytosis and the activity of intracellular proteins. Additionally, under pathological conditions or *in vitro* stimulation, neutrophils can release web-like structures known as neutrophil extracellular traps (NETs) (1).

NETs contain various proteins, including myeloperoxidase (MPO), cathepsin G, neutrophil defensins, and the calprotectin protein complex (S100A8/A9). Among these proteins, MPO consists of two subunits: a 59-kDa heavy subunit and a 13.5-kDa light subunit, linked by covalent bonds associated with the heme group. The mature MPO protein is a glycosylated 146-kDa homodimer, found in the primary granules of neutrophils and lysosomes of monocytes (2).

MPO serves as a valuable marker for many diseases, including acute myelogenous leukemia, cardiovascular disease, Alzheimer's disease, multiple sclerosis, stroke, Parkinson's Disease, depression, traumatic brain injury, epilepsy, and rheumatoid arthritis (3).

To investigate the roles of MPO in mouse inflammatory disease models, such as arthritis (4) and experimental autoimmune encephalomyelitis (5), Chondrex, Inc. offers a highly sensitive and specific Mouse MPO Detection ELISA Kit (Cat # 6051). This kit enables the quantitative measurement of MPO in mouse serum, plasma, and other biological samples. Utilizing the sandwich ELISA principle, it employs two monoclonal antibodies specific to target mouse MPO.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse MPO (60511)	1 vial	50 ng, lyophilized	-20°C
Anti-MPO Capture Antibody (60512)	1 vial	100 µl, 1 mg/ml	-20°C
Anti-MPO Detection Antibody (60513)	1 vial	100 µl	-20°C
Solution A - Coating Buffer (9052)	1 bottle	10 ml	-20°C
Solution D – Blocking/Sample/Standard/Detection Antibody/Streptavidin Peroxidase Dilution Buffer (9055)	2 bottles	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB Solution (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
ELISA Plate	1 each	96-well (8-well strips x 12)	-20°C

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ASSAY OUTLINE

Add 100 µl of capture antibody solution into wells

↓ Incubate at at 4ºC overnight.
Wash plate.

Add 100 µl of blocking buffer into wells

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of diluted standards and samples into wells

↓ Incubate at room temperature for 3 hours.
Wash plate.

Add 100 µl of diluted detection antibody Solution

↓ Incubate at room temperature for 1 hours.
Wash plate.

Add 100 µl of diluted streptavidin peroxidase solution

↓ Incubate at room temperature for 30 minutes.
Wash plate.

Add 100 µl of TMB solution

↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

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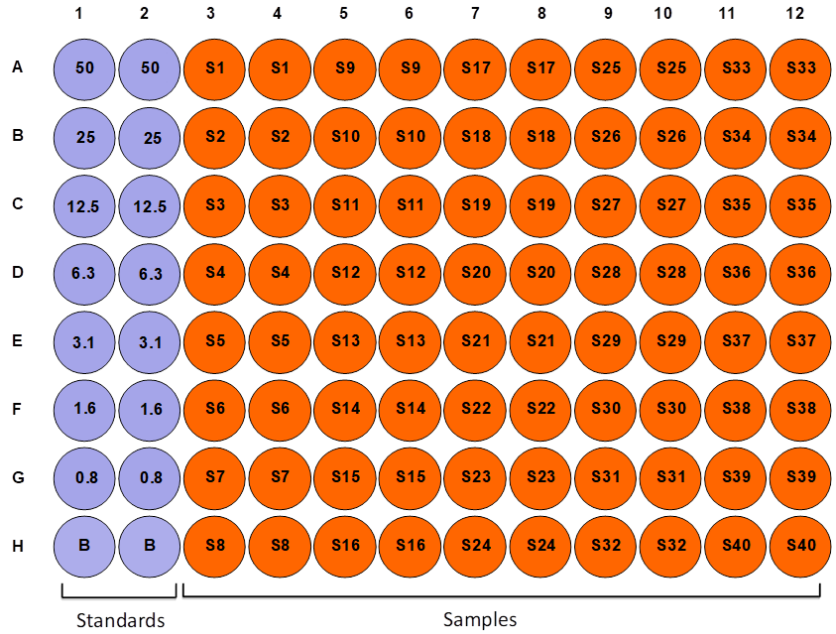
PLATE MAPPING



NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

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ASSAY PROCEDURE

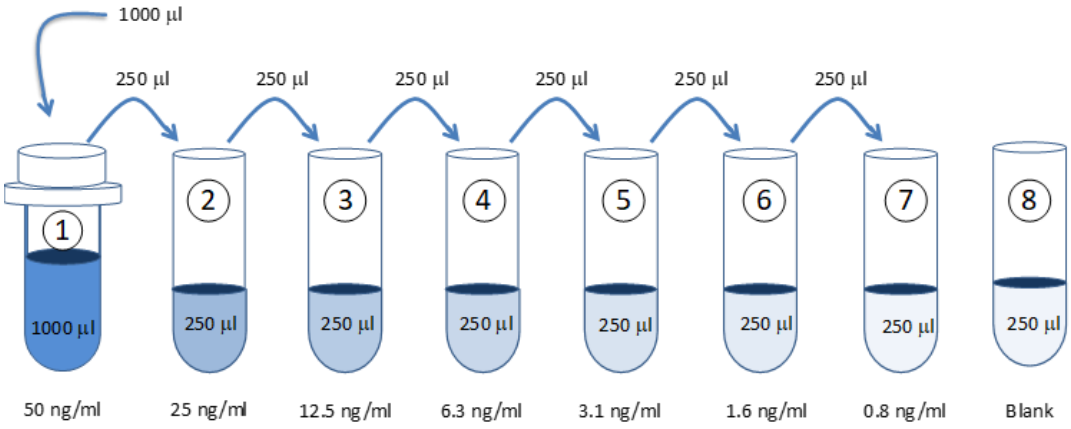
1. **Prepare Capture Antibody Solution:** Dilute one vial of capture antibody with 10 ml of Coating Buffer (Solution A). Alternatively, dilute according to the table below. Add 100 µl of capture antibody solution to each well and incubate at 4ºC overnight. Any leftover capture antibody stock solution may be stored at -20ºC for future assays.

Strip #	Capture Antibody (µl)	Solution A (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

2. **Wash**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

3. **Block Plate**: Add 100 µl of Solution D to each well and incubate at room temperature for 1 hour.

4. **Prepare Standard Dilutions**: The recommended standard range is 0.8 - 50 ng/ml. Dissolve one vial of Standard (50 ng/vial) in 1 ml of Solution D and keep it as standard stock. Then serially dilute it with Solution D. For example, mix 250 µl of the 50 ng/ml solution with an equal volume of Solution D to make a 25 ng/ml solution, and then repeat it five more times for 12.5, 6.3, 3.1, 1.6, and 0.8 ng/ml standard solutions.



5. **Prepare Sample Dilutions**: Dilute samples at least 1:1 with Solution D depending on the estimated MPO level in the samples. Two or three different sample dilutions are recommended if the MPO levels in the samples are unknown.

6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Standards and Samples**: Add 100 µl of standards, Solution D (blank), and samples to wells in duplicate. Incubate at room temperature for 3 hours.

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

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9. **Add Detection Antibody**: Dilute one vial of detection antibody in 10 ml Solution D or alternatively, dilute as shown in the following table. Add 100 µl of anti-MPO detection antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Detection Antibody (µl)	Solution D (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

10. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

11. **Add Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Solution D as shown in the following table. Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

12. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

13. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

14. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

15. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

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CALCULATING RESULTS

1. Average the duplicate OD values for the standards, blanks (B), and test samples.

2. Subtract the averaged blank OD values from the averaged OD values of the standards and test samples.

3. Plot the OD values of the standards against the ng/ml of mouse MPO standard. Using a log/log plot will linearize the data. Figure 1 shows an example of a standard curve where the standard range is 0.8 to 50 ng/ml.

4. The ng/ml of MPO in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the MPO concentration (ng/ml) in the original test samples.

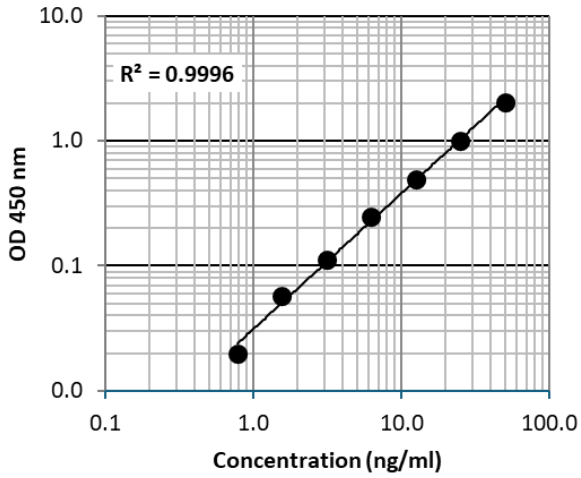

Figure 1 - A Typical Standard Curve for the Mouse MPO Detection ELISA Kit

VALIDATION DATA

Table 1 - Reproducibility Data for the Mouse MPO Detection ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	6.1	4.1	3.0
Inter-Assay CV (%)	7.5	7.3	7.2
Spike Test* (%)	93%	95%	93%

*Known amounts of MPO were added to samples and then diluted with Sample/Standard Dilution Buffer (Solution D) to assay MPO by ELISA.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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