Product Manual

Myeloperoxidase (human), ELISA kit

Catalog #: ADI-900-115

96 Well Kit

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

DESCRIPTION

The Myeloperoxidase (human), ELISA kit is a complete kit for the quantitative determination of MPO in biological fluids. Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to MPO immobilized on a microtiter plate to bind the MPO in the standards or sample. A native MPO Standard is provided in the kit. After a short incubation, the excess sample or standard is washed out and a rabbit polyclonal antibody to MPO is added. This antibody binds to the MPO captured on the plate. After a short incubation, the excess antibody is washed out and goat anti-rabbit IgG conjugated to horseradish peroxidase is added, which binds to the polyclonal MPO antibody. Excess conjugate is washed out and substrate is added. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450nm. The measured optical density is directly proportional to the concentration of MPO in either standards or samples. For further explanation of the principles and practices of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

INTRODUCTION

Myeloperoxidase (MPO) is synthesized as a single chain precursor, which is then processed into a tetramer consisting of 2 light chains and 2 heavy chains. In response to microbial invasion, MPO is released from the cytoplasmic granules of neutrophils into the extracellular space and phagosome, where it generates hypohalous acids that have a strong microbicidal effect[3]. Since myeloperoxidase is a major component of myeloid precursor cells, the enzymatic activity of MPO is used to classify acute leukemias[4]. Myeloperoxidase is used also as a marker of airway inflammation caused either by disease, such as asthma[5-6], or environmental irritants[7]. In addition to the protein itself, autoantibodies against MPO are markers for several systemic vasculitis diseases[8].

SAFETY WARNINGS & PRECAUTIONS

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- Stop Solution 2 is a 1N hydrochloric acid solution. This solution is caustic; care should be taken in use.
- The activity of the horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.
- We test this kit's performance with a variety of buffers, however it is possible that high levels of interfering substances may cause variation in assay results.
- The MPO Standard provided, Catalog No. 80-1090, should be handled with care because of the known and unknown effects of MPO.
- **Caution: The MPO standard is derived from human serum. Treat as a biohazard.**

MATERIALS SUPPLIED

1. **human MPO Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-1092**

   A plate using break-apart strips coated with a mouse monoclonal antibody specific to MPO.

2. **human MPO Antibody, 10ml, Catalog No. 80-1445**

   A yellow solution of rabbit polyclonal antibody to MPO.

3. **Assay Buffer 13, 55ml, Catalog No. 80-1500**

   Tris buffered saline containing proteins and detergents.

4. **Assay Buffer 31 Concentrate, 55ml, Catalog No. 80-1625**

   Citrate buffered saline containing detergents.

5. **human MPO Conjugate, 10ml, Catalog No. 80-1093**

   A blue solution of goat anti-rabbit IgG conjugated to Horseradish peroxidase.

6. **Wash Buffer Concentrate, 100ml, Catalog No. 80-1287**

   Tris buffered saline containing detergents.

7. **human MPO Standard, 0.25ml, Catalog No. 80-1090**

   A solution of 125ng/ml MPO from human neutrophils.

8. **TMB Substrate, 10ml, Catalog No. 80-0350**

   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. Protect from prolonged exposure to light.

9. **Stop Solution 2, 10ml, Catalog No. 80-0377**

   A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: Caustic.

10. **MPO (human) Assay Layout Sheet, 1 each, Catalog No. 30-0188**

11. **Plate Sealer, 3 each, Catalog No. 30-0012**

STORAGE

All components of this kit are stable at 4°C until the kit's expiration date.

OTHER MATERIALS NEEDED

1. Deionized or distilled water.
2. Phenylmethylsulfonyl fluoride (PMSF), Sigma #P7626 or equivalent.
3. Protease inhibitor cocktail (PIC), Sigma #P8340 or equivalent.
4. Precision pipets for volumes between 100µl and 1,000µl.
5. Repeater pipet for dispensing 100µl.
6. Disposable beakers for diluting buffer concentrates.
7. Graduated cylinders.
8. A microplate shaker.
9. Adsorbent paper for blotting.
10. Microplate reader capable of reading at 450nm, preferably with correction between 570nm and 590nm.
11. Graph paper for plotting the standard curve.

SAMPLE HANDLING

The ELISA is compatible with human MPO samples in sputum and nasal lavage supernatants, **lithium heparin plasma**, culture supernates, urine, and other standard buffers. Samples diluted sufficiently into the appropriate assay buffer plus inhibitors (see Reagent Preparation, page 8, #2) can be read directly from a standard curve. Two separate assay buffers are supplied with this kit. Assay Buffer 13 is for use with sputum, nasal lavage supernates, and urine. Lithium heparin plasma samples must be diluted with Assay Buffer 31 only. Please refer to the Sample Recovery recommendations on page 15 for details of suggested dilutions. **Serum, sodium heparin plasma, and EDTA plasma samples are not recommended for use in this assay as low recoveries will be obtained**.

Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of assay buffer. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or the appropriate assay buffer to calculate concentrations of human MPO in the appropriate matrices. **If the end user chooses to use their own assay buffer, it is up to the end user to determine the appropriate dilution of samples and assay validation.**

Reported sample handling for other matrices:

Tissue samples should be homogenized in a phosphate buffer containing 1% hexadecyltrimethylammonium hydroxide. Samples should then be centrifuged at 4,500 x g for 15 minutes at 4°C to assay MPO in the supernatants.

Bronchoalveolar Lavage fluid (BALF) may need to be concentrated prior to use in assay. Cell-free BALF can be subjected to pressure filtration under nitrogen to achieve this.

PROCEDURAL NOTES

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards must be made up in polypropylene tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
8. **Prior to addition of antibody, conjugate and substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results**.

REAGENT PREPARATION

1. Wash Buffer

Prepare the Wash Buffer by diluting 50ml of the supplied concentrate with 950ml of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. Assay Buffer plus Inhibitors

Assay Buffer 13: For use with sputum, nasal lavage supernatants, and urine samples.

Immediately prior to use in the assay, PMSF and PIC must be added to the buffer. If using Sigma Protease Inhibitor Cocktail #P8340, add 0.5µl/ml PIC or equivalent concentration according to alternate vendor's specification sheet. Add PMSF to a final concentration of 1mM. This modified Assay Buffer 13 must be used for standard reconstitution and all sample and standard dilutions to ensure optimal integrity of MPO. Fresh Assay Buffer 13 plus inhibitors must be prepared for each assay.

Assay Buffer 31: For use with lithium heparin plasma samples.

Dilute Assay Buffer 31 Concentrate (10X concentrate) to a final volume of 1X by adding 1mL concentrated buffer to 9mLs ddH_2O. Immediately prior to use in the assay, PMSF and PIC must be added to the diluted buffer. If using Sigma Protease Inhibitor Cocktail #P8340, add 0.5µl/ml PIC or equivalent concentration according to alternate vendor's specification sheet. Add PMSF to a final concentration of 1 mM. This modified Assay Buffer 31 must be used for standard reconstitution and all plasma samples and standard dilutions to ensure optimal integrity of MPO. Fresh Assay Buffer 31 plus inhibitors must be prepared for each assay.

3. MPO Standards

Allow the human MPO standard solution to warm to room temperature. Label seven 12x75mm polypropylene tubes #1 through #7. Pipet 900µl of the appropriate standard diluent into tube #1. Pipet 500µl of standard diluent into tubes #2 though #7. Add 100µl of the 125ng/ml Standard to tube #1. Vortex thoroughly. Add 500µl of tube #1 into tube #2 and vortex. Add 500µl of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #7.

The concentration of human MPO in tubes #1 through #7 will be 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, and 0.195ng/ml respectively. See MPO Assay Layout Sheet for dilution details.

Diluted standards should be used within 30 minutes of preparation.

ASSAY PROCEDURE

Bring all reagents to room temperature for at least 30 minutes prior to opening. All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4°C.
2. Pipet 100µl of standard diluent (Assay Buffer 13 plus inhibitors , Assay Buffer 31 plus inhibitors, or tissue culture media) into the S0 (0ng/ml standard) wells.
3. Pipet 100µl of Standards #1 through #7 into the appropriate wells.
4. Pipet 100µl of the Samples into the appropriate wells.
5. Tap the plate gently to mix the contents.
6. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500rpm.
7. Empty the contents of the wells and wash by adding 400µl of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
8. Pipet 100µl of yellow Antibody into each well, except the Blank.
9. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500rpm.
10. Empty the contents of the wells and wash by adding 400µl of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
11. Add 100µl of blue Conjugate to each well, except the Blank.
12. Seal the plate and incubate at room temperature on a plate shaker for 30 minutes at ~500rpm.

13. Empty the contents of the wells and wash by adding 400µl of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
14. Pipet 100µl of Substrate Solution into each well.
15. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker at ~500rpm.
16. Pipet 100µl Stop Solution 2 to each well.
17. Blank the plate reader against the Blank wells, read the optical density at 450nm, preferably with correction between 570 and 590nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of human MPO in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of human MPO can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average Blank OD

2. Plot the Average Net OD for each standard versus human MPO concentration in each standard. Approximate a straight line through the points. The concentration of human MPO in the unknowns can be determined by interpolation.

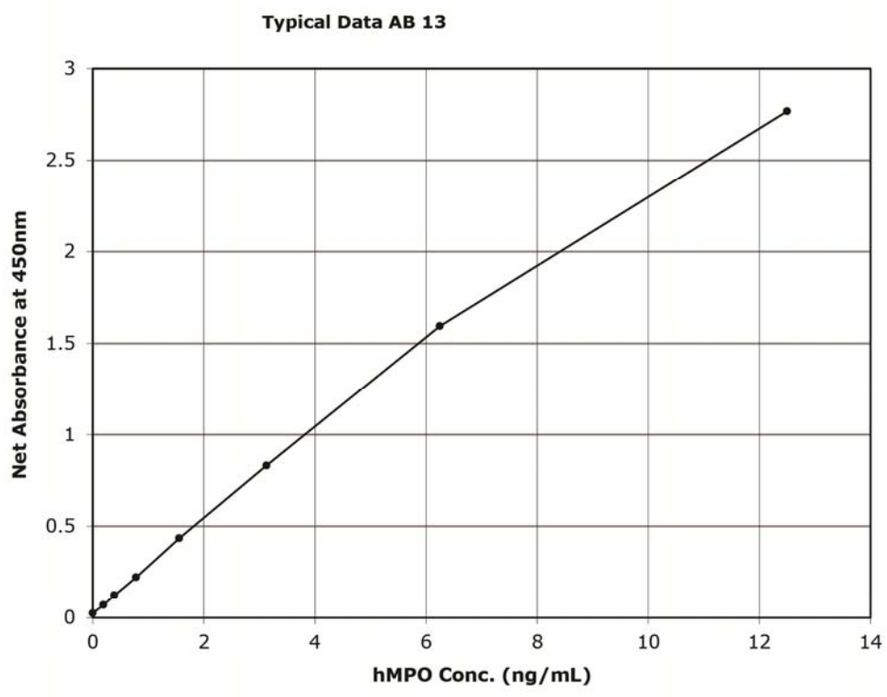
TYPICAL RESULTS IN ASSAY BUFFER 13

The results shown below are for illustration only and **should not** be used to calculate results.

Sample	Net OD	Net OD
S0	0.024	**0**
S1	2.712	**12.5**
S2	1.551	**6.25**
S3	0.820	**3.13**
S4	0.428	**1.56**
S5	0.224	**0.78**
S6	0.123	**0.39**
S7	0.074	**0.195**
Unknown 1	2.283	**9.979**
Unknown 2	0.277	**0.990**

TYPICAL STANDARD CURVE FOR ASSAY BUFFER 13

A typical standard curve is shown below. This curve **must not** be used to calculate MPO concentrations; each user must run a standard curve for each assay.



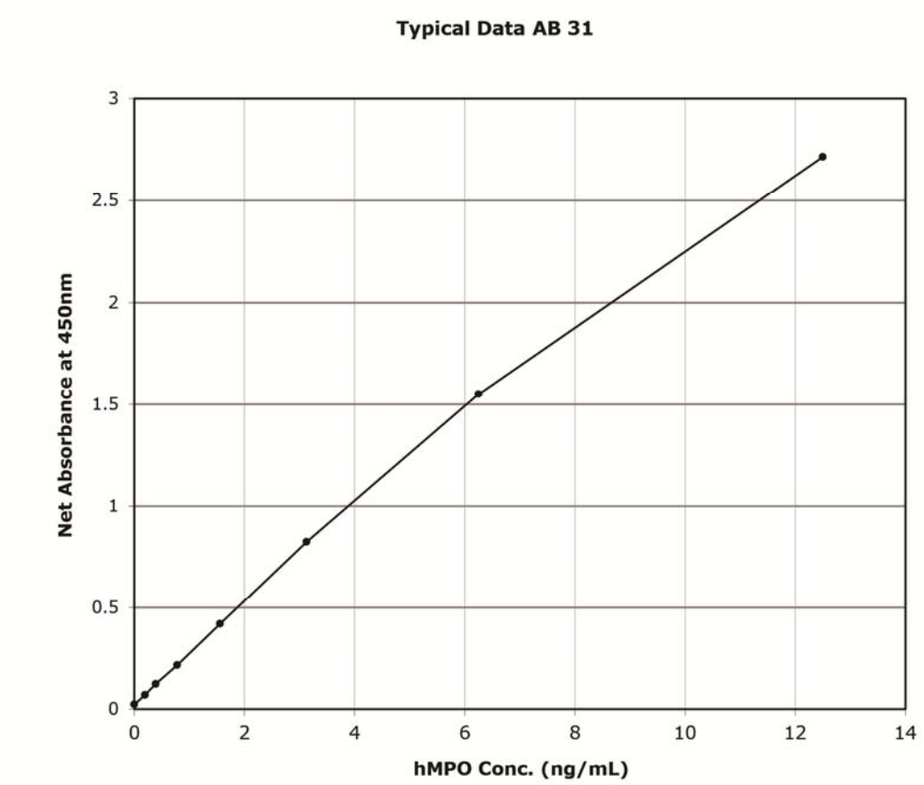
TYPICAL RESULTS IN ASSAY BUFFER 31

The results shown below are for illustration only and **should not** be used to calculate results.

Sample	Net OD	Net OD
S0	0.023	0
S1	2.712	**12.5**
S2	1.551	**6.25**
S3	0.825	**3.13**
S4	0.420	**1.56**
S5	0.217	**0.78**
S6	0.124	**0.39**
S7	0.070	**0.195**
Unknown 1	2.299	**10.042**
Unknown 2	0.256	**0.922**

TYPICAL STANDARD CURVE FOR ASSAY BUFFER 31

A typical standard curve is shown below. This curve **must not** be used to calculate MPO concentrations; each user must run a standard curve for each assay.



PERFORMANCE CHARACTERISTICS

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[13].

Sensitivity

The sensitivity of the assay, defined as the concentration of human MPO measured at 2 standard deviations from the mean of 24 zeros along the standard curve, was determined to be 0.028ng/ml in Assay Buffer 13, and 0.019ng/ml in Assay Buffer 31.

Linearity

A buffer sample containing human MPO was serially diluted 1:2 in Assay Buffer 13 and measured in the assay. The results are shown in the table below.

Dilution	Expected Conc.	Observed Conc.	Recovery (%)
Neat	————	9.536ng/ml	————
1:2	4.768ng/ml	4.927ng/ml	103.3 %
1:4	2.384ng/ml	2.467ng/ml	103.5 %
1:8	1.192ng/ml	1.208ng/ml	101.3 %
1:16	0.596ng/ml	0.618ng/ml	103.7 %
1:32	0.298ng/ml	0.304ng/ml	102.8 %

A buffer sample containing human MPO was serially diluted 1:2 in Assay Buffer 31 and measured in the assay. The results are shown in the table below.

Dilution	Expected Conc.	Observed Conc.	Recovery (%)
Neat	————	10.067ng/ml	————
1:2	5.034ng/ml	4.897ng/ml	97.3 %
1:4	2.517ng/ml	2.444ng/ml	97.1 %
1:8	1.258ng/ml	1.269ng/ml	100.8 %
1:16	0.629ng/ml	0.630ng/ml	100.1 %
1:32	0.315ng/ml	0.297ng/ml	94.4 %

Precision

Intra-assay precision was determined by assaying 24 replicates of three buffer controls containing human MPO in a single assay.

In Assay Buffer 13		In Assay Buffer 31	
ng/ml	%CV	ng/ml	%CV
9.6	1.1	10.3	1.6
2.0	1.3	2.0	1.9
1.0	3.6	.0	3.6

Inter-assay precision was determined by measuring buffer controls of varying human MPO concentrations in multiple assays over several days.

In Assay Buffer 13		In Assay Buffer 31	
ng/ml	%CV	ng/ml	%CV
9.9	3.5	10.0	1.9
2.0	3.5	1.9	3.6
0.98	3.1	0.95	7.2

Cross Reactivities

The cross reactivities for a number of related compounds was determined by dissolving the cross reactant in Assay Buffer 13 plus inhibitors. These samples were then measured in the MPO assay and the measured MPO concentration calculated. The percent cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity
human Myeloperoxidase	100%
human Erythropoietin	1.1%
bovine Lactoperoxidase	0.4%
human Elastase	0.2%
human Lactoferrin	0.2%
sheep COX-2	0.1%
human Thyroid Peroxidase	< 0.1%
human Eosinophil-derived Neurotoxin	< 0.1%

SAMPLE RECOVERIES

Please refer to pages 6-8 for Sample Handling recommendations and Standard preparation.

Human MPO concentrations were measured in tissue culture media, sputum supernatant, nasal lavage supernatant, lithium heparin plasma, and urine. Undiluted samples of these matrices were spiked with human MPO, and then diluted with the appropriate assay buffer plus inhibitors and assayed in the kit. The following results were obtained:

Sample	% Recovery*	Recommended Dilution*
Tissue Culture Media	95.0	none
Sputum Supernatant	101.0	≥1:16
Nasal Lavage Supernatant	98.8	≥1:2
Lithium Heparin Plasma	105.0	1:24
Urine	93.2	≥1:8

* See Sample Handling instructions on page 6 for details.

****WARNING: If the end user chooses to not use the provided assay buffer(s), it is up the end user to determine the appropriate dilution of samples and assay validation for their chosen buffer.**

REFERENCES

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NOTES

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