HUMAN MYELOPEROXIDASE (MPO) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN MPO CONCENTRATIONS IN PLASMA, SERUM AND SALIVA.

PURCHASE INFORMATION:

ELISA NAME	HUMAN MPO ELISA
Standard range	0.5 - 32 ng/mL
Sensitivity	100 pg/ml
Sample Volume	100 µl
Sample Type	Serum, plasma, cell culture or tissue
Specificity	Human MPO only
Dilution Factor	10 (Optimal dilutions should be determined by each laboratory for each application)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2°C - 8°C

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Human MPO immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human MPO in cell culture supernates, serum, and plasma. It contains recombinant human MPO and antibodies raised against this protein. It has been shown to accurately quantify recombinant human MPO. Results obtained with naturally occurring MPO samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human MPO.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for MPO has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MPO present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for MPO is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is add to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of MPO bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
MPO Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against MPO.	172-02-01	1 plate
MPO Standard – 32ng/vial of recombinant human MPO in a buffered protein base with preservatives; lyophilized.	172-02-02	1 vial
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of Biotinylated antibody against MPO with preservatives; lyophilized.	172-02-03	1 vial
Positive Control – one vial of recombinant human MPO, lyophilized	172-02-04	1 vial
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugated to HRP	SAHRP	1 vial
Dilution Buffer – 60mL/vial of buffered protein based solution with preservatives	DB01	1 vial
Wash Buffer – 50 mL/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
TMB Substrate Solution – 11 mL /vial of TMB substrate solution	TMB01	1 vial
Stop Solution – 11 mL/vial of 0.5N HCl	S-STOP	1 vial
Plate Sealer	EAPS	1 piece

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should

be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard, Antibody Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months. Reconstituted Positive Control should be prepared and used immediately.

Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

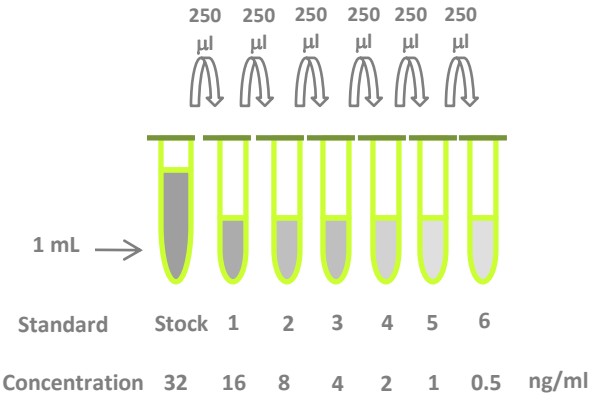
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

MPO Standard - Refer to vial label for reconstitution volume. Reconstitute the **MPO** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 32 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (see below). Mix each tube thoroughly before the next transfer. The 32 ng/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1000 µl	32 ng/ml
# 1	250 µl of stock	250 µl	16 ng/ml
# 2	250 µl of 1	250 µl	8 ng/ml
# 3	250 µl of 2	250 µl	4 ng/ml
# 4	250 µl of 3	250 µl	2 ng/ml
# 5	250 µl of 4	250 µl	1 ng/ml
# 6	250 µl of 5	250 µl	0.5 ng/ml



Positive Control – Reconstitute the **Positive Control** with 1.0 mL Dilution Buffer. *Positive Control should be prepared and used immediately*.

Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. *Note: 1 x*

working solution of Streptavidin-HRP Conjugate should be used within a few days.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (A2, A3).
4. Add 100 µL of Standard (from B2,B3 to G2,G3 and G4,G5), sample, or control (F4,F5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate** working solution to each well. Incubate for 45 minutes on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 5-10 minutes at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 30 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the MPO concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.
All samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified human MPO.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of MPO was 100 pg/mL.

TYPICAL DATA

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

MPO (NG/ML)	AVERAGE OD450 (CORRECTED)*
0.5	0.044
1	0.113
2	0.215
4	0.429
8	0.704
16	1.441
32	2.521

SPECIFICITY

ADIOKINES	CROSSREACTIVITY (%)
Human MPO	100
Human CRP	0
Human RBP-4	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µL of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Detection Antibody to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Streptatvin HRP conjugate to each well. Incubate 10 minutes on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate to each well. Incubate 5-10 minutes on the bench top. Protect from light.
↓
Add 100 µL Stop Solution to each well. Read 450nm within 15 minutes