HUMAN MYELOPEROXIDASE (MPO) ELISA KIT

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

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN MPO ELISA KIT**
Catalog No.	SK00172-06
Lot No.	
Formulation	96 T
Standard range	125 – 8000 pg/mL
Sensitivity	30 pg/mL
Sample Volume	100 µL
Sample Type	Serum, EDTA Plasma, Saliva
Dilution Factor	**10 -20 (Optimal dilutions should be determined by each laboratory for each application)**
Specificity	Human MPO
Calibration	Human MPO
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8° C for 4 months. Check page 2-3 for detail
This kit contains sufficient materials to run 35-40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human MYELOPEROXIDASE (MPO) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human MPO from serum, plasma and Saliva in a sandwich ELISA format.

This immunoassay contains recombinant human MPO and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural MPO samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human MPO. The capture antibody can bind to the human MPO in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human MPO is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human MPO bound in the standard dilutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
MPO Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against MPO.	**172-06 - 01**	**1 plate**
MPO Standard – 8ng per vial of recombinant human MPO in a buffered protein base with preservative; lyophilized.	**172-06 - 02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL of 10-fold concentrate of biotinylated antibody against MPO with preservative; lyophilized.	**172-06 - 03**	**1 vial**
Positive Control - one vial of recombinant human MPO; lyophilized. (optional)	**172-06 - 04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate, with preservative.	**SAHRP**	**1 vial**
Standard Reconstitute Solution – 0.5 mL of solution.	**DB17**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB03**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB15**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution -11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**

Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 4 months. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2-8 °C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

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

SAMPLE PREPARATION

Serum or plasma samples may require 10 ~ 20 fold dilution.

Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

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 1x Wash Buffer.

*The lyophilized **Standard 172-06-HS-02** and **Positive Control 172-06-HS-04** should be centrifuged at 5000~ 10000 rpm for 1 min and reconstituted with **50 µL of Standard Reconstitute Solution** for a minimum 3 ~ 5 minutes with gentle agitation. Then add 0.95 mL of Dilution Buffer DB08B as stock solution of standard or positive control.

MPO Standard* - Reconstitute the lyophilized MPO Standard with **50 µL of Standard Reconstitute Solution.** Allow the standard to sit for a minimum of 3-5 minutes with gentle agitation. Then add 0.95 mL of Dilution Buffer DB08B as standard stock solution. 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 (below). Mix each tube thoroughly before the next transfer. The **8000 pg/mL** standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL). Store the standard stock at -20 ~ - 70°C for few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1 mL*	8000 pg/ml
# 1	250 µl of stock	250 µl	4000 pg/ml
# 2	250 µl of 1	250 µl	2000 pg/ml
# 3	250 µl of 2	250 µl	1000 pg/ml
# 4	250 µl of 3	250 µl	500 pg/ml
# 5	250 µl of 4	250 µl	250 pg/ml
# 6	250 µl of 5	250 µl	125 pg/ml

Positive Control*– Reconstitute the Positive Control with **50 µL of Standard Reconstitute Solution.** Allow the Positive Control to sit for a minimum of 3-5 minutes with gentle agitation. Then add 0.95 mL of **Dilution Buffer DB08B** to prepare the working

solution of positive control. Discard the positive control after use. It is for one time use only.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 ml of **Antibody Diluent Solution (DB15)** to produce a 10-fold concentrated stock solution. For 96 wells test, freshly pipette 9.45 mL of **Antibody Diluent Solution (DB15)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. For a partial strip test, freshly prepare 900 µL per strip of working solution. Store the stock solution of 10-fold concentrated detection antibody at -20 °C for a few days.

Streptavidin-HRP Conjugate – For 96 wells test, freshly pipette 11.88 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light).** *The working solution should be used in 20- 30 min. For a partial strip test, freshly prepare 900 µL per strip of working solution. Store the stock solution of 100-fold concentrated Streptavidin HRP at 2 -8 °C for 10 months.*

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL of Dilution Buffer to Blank wells.
3. Add 100 µL of Standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x 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.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 90 minutes on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 3- 7 min on a microplate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 3 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

MPO (pg/mL)	Average OD450nm (Corrected)
Blank	0 (0.110)
125	0.062
250	0.130
500	0.264
1000	0.508
2000	1.046
4000	2.019
8000	2.997

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human MPO	100
Human PON1	0
Mouse MPO	0

Human MPO Recombinant derived from E. Coli or sf21 cells may not be detected by this ELISA Kit.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 90 minutes on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 45 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate for 3 -7 min on plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 minutes.

The research samples were diluted by Dilution Buffer DB08B. The linearity and recovery was assayed by MPO (Human) ELISA Kit SK00172-06

Sample Type	Dilution Factor	Assayed (pg/mL)	Final (ng/mL)	Recovery (%)
Human Serum	5 X	3076.496	15.382	100
Human Serum	10 X	1490.271	14.903	96.8
Human Serum	20 X	697.188	13.944	90.6
Human Plasma	10 X	4148.370	41.484	100
Human Plasma	20 X	2144.971	42.889	103
Human Plasma	40 X	1043.171	41.727	100
Human Saliva	2 X	6289.740	12.579	100
Human Saliva	4 X	3058.928	12.235	97.2
Human Saliva	8 X	1501.042	12.008	95.4