

# **MSD<sup>®</sup> Human Myeloperoxidase Assay**

MULTI-ARRAY<sup>®</sup> 96-well Small Spot Plates

## ***Application***

For the quantitative measurement of Myeloperoxidase in human serum and plasma.

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Storage

## ***MSD Materials***

<input type="checkbox"/> Read Buffer T (4X)	RT
<input type="checkbox"/> Blocker A Kit	4 °C
<input type="checkbox"/> MULTI-ARRAY 96-well Small Spot Human MPO plate	4 °C
<input type="checkbox"/> SULFO-TAG <sup>™</sup> Human MPO Detection Antibody (500 µg/mL)	4 °C
<input type="checkbox"/> Human MPO Assay Diluent	-20 °C
<input type="checkbox"/> MPO Calibrator (1 µg/mL)	-20 °C

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## ***Other Materials & Equipment (not supplied)***

- Deionized water for diluting Read Buffer
- Phosphate buffered saline for plate washing.
- Plate washer or other efficient multi-channel pipetting equipment for washing 96 well plates
- Appropriate liquid handling equipment that must accurately dispense 25 and 150 µL into a 96-well micro plate

## Protocol at a Glance

### Notes:

**The protocol can be completed in approximately 3 hours** if each reagent is prepared during the preceding incubation. This time can be reduced to **2 hours if the blocking reagent is added the night before**. All reagents can be prepared hours ahead of time if desired.

*Read the entire detailed instructions before beginning work.*

1. Block plates for 1 hour at room temperature (alternatively, block plates overnight at 4 °C).
2. Wash.
3. Add Assay Diluent followed by calibrator or diluted sample and incubate for 1 hour with shaking.
4. Wash.
5. Add Detection Antibody Reagent and incubate for 1 hour with shaking.
6. Wash.
7. Add Read Buffer and analyze immediately.

## Preparation Instructions

### Prepare Blocker A Kit:

1. Prepare Blocker A Solution following the instructions included in the Blocker A Kit.

### Prepare Calibrators:

1. MSD recommends the preparation of an 8-point calibration curve consisting of at least 2 replicates of each point. Each well will require 10 µL of calibrator. Thaw the MPO Assay Diluent and MPO Calibrator (1 µg/mL) and prepare serial dilutions of calibrator stock in Assay Diluent.  
*A recommended calibrator dilution procedure is listed below, for preparing three replicates of each calibrator.*
  - Prepare 200 µL of a high calibrator containing 50 ng/mL of MPO by adding 10 µL of the supplied calibrator stock (1 µg/mL) to 190 µL of Assay Diluent.
  - Prepare 6 serial dilutions starting with the 50 ng/mL high calibrator and diluting by a factor of 5; add 10 µL of the higher calibrator to 40 µL of Assay Diluent.
  - This will create seven calibrators with 50, 10, 2, 0.40, 0.08, 0.016, 0.0032 ng/mL of MPO.
  - Reserve 50 µL of Assay Diluent as the 8<sup>th</sup> (zero) calibrator.
  - **NOTE: The sample requires a 20X dilution. The calibrators are not diluted prior to their addition to the plate. Therefore, when analyzing data, multiply the calibrator concentrations by 20 so that the sample concentrations can be read directly from the calibration curve. For example, the dilution-corrected high MPO calibrator is 1000 ng/mL.**
2. Calibrators should be kept at 4 °C (for up to 4 hours) if not used immediately. The Assay Diluent is stable for one week at 4 °C. For

longer storage, aliquot and store at -20 °C. Assay Diluent may be refrozen twice.

#### **Prepare Samples:**

1. Dilute samples 1:20 in Assay Diluent. For example, add 10 µL of sample to 190 µL of Assay Diluent and mix thoroughly. Each replicate will require 10 µL of diluted sample.

#### **Prepare Assay Diluent:**

1. Determine total number of wells in the experiment. Each well will require 40 µL of Human MPO Assay Diluent.
2. Thaw enough diluent for the experiment – the reagent is stable at room temperature for a few hours. Assay Diluent is stable at 4 °C for one week. For longer storage, aliquot and store at -20 °C. Assay diluent may be refrozen twice.

#### **Prepare Detection Antibody Reagent:**

1. Determine total number of wells in the experiment. Each well will require 25 µL of Detection Antibody Reagent.
2. Prepare Detection Antibody Reagent by diluting SULFO-TAG MPO Detection Antibody (500 µg/mL) in MPO Assay Diluent to a concentration of 4 µg/mL (1:125).
3. Detection Antibody Reagent should be kept at 4 °C until used (up to 4 hours).

#### **Dilute Read Buffer:**

1. Determine total number of wells in experiment. Each well will receive 150 µL of Read Buffer T. Prepare an extra 20%.
2. Dilute 4X Read Buffer T to 1X with deionized water.
3. Diluted Read Buffer may be stored at room temperature for later use.

### ***Assay Protocol***

Begin with a MULTI-ARRAY 96 well Small Spot Human MPO plate.

1. Add 200 µL/well of Blocker A Solution and incubate at room temperature for 1 hour or overnight at 4 °C.
2. Wash plates 3 times with phosphate buffered saline (PBS).
3. Add 40 µL/well of MPO Assay Diluent.
4. Add 10 µL/well calibrator or diluted sample and incubate at room temperature with shaking for 1 hour.
5. Wash plates 3 times with PBS.

6. Add 25  $\mu\text{L}$ /well Detection Antibody Reagent and incubate at room temperature with shaking for 1 hour.
7. Wash plates 3 times with PBS.
8. Prepare SECTOR™ instrument so that the plate can be read immediately after Read Buffer addition.
9. Add 150  $\mu\text{L}$ /well 1X Read Buffer T. *Avoid bubbles.* An electronic multi-pipettor at moderate speed setting is recommended.
10. Analyze immediately with SECTOR instrument.

**Notes:**

*Note that bubbles introduced to the well during Read Buffer addition will interfere with reliable imaging of the plate.*

