TECHNICAL MANUAL

Myeloperoxidase (MPO) Peroxidation Activity Fluorometric Assay Kit

- **SKU CODE:** MAES0004
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Add inhibitor to control wells
- Add reaction working solution
- Incubate at 37°C
- Add inhibitor to sample and standard wells
- Measure fluorescence intensity

2. Intended use

This kit can be used to detect myeloperoxidase (MPO) peroxidation activity in serum, plasma, and tissue samples.

3. Detection principle

Under the catalysis of peroxidase, hydrogen peroxide can oxidize the non-fluorescent probe into a fluorescent substance, and its fluorescence intensity is proportional to the total peroxidase activity in the sample. This kit specifically inhibits the peroxidase activity of MPO in the sample through an MPO enzyme inhibitor, thus distinguishing the peroxidase activity of MPO in the sample from that of other peroxidases.

Hydrogen peroxide + Substrate → Fluorescence value F_2 (Ex/Em=535 nm/587 nm)

Hydrogen peroxide + Substrate + MPO inhibitor → Fluorescence value F_1

Fluorescence value of myeloperoxidase peroxidation activity = Fluorescence value F_2 - Fluorescence value F_1

4. Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	60 mL × 5 vials	-20°C, 12 months

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 2	Probe	0.25 mL × 1 vial	1.25 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 3	Substrate	0.25 mL × 1 vial	1.25 mL × 1 vial	-20°C, 12 months
Reagent 4	Inhibitor	1.2 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months
Reagent 5	25 μmol/L Resorufin Standard	1.5 mL × 1 vial	7.5 mL × 1 vial	-20°C, 12 months, protect from light
	Black Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), micropipette, vortex mixer, water bath

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. Preheat the buffer solution at 37°C for 20 min and use only after it is completely clarified.
3. **Preparation of reaction working solution::** For each well, prepare 40 μL of reaction working solution by mixing 36 μL of buffer solution, 2 μL of probe, and 2 μL of substrate. The reaction working solution should be prepared immediately before use and protected from light.
4. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 25 μmol/L resorufin standard solution with buffer solution to create a serial concentration. The recommended dilution gradient is: 0, 2, 4, 6, 8, 10, 12, 15 μmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL buffer solution with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

- Porcine serum: 5-10

- Rabbit serum: 3-5

- Rat serum: 2-5

- Mouse serum: 10-20

- Mouse plasma: 30-50

- Horse serum: 2-5

- 10% Rat heart tissue homogenate: 1

- 10% Rat lung tissue homogenate: 1

Note: The diluent is buffer solution. For the dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. The key points of the assay

1. Dilute the samples to the optimal concentration for detection if the MPO peroxide activity of samples exceeds the detection range.
2. The prepared reaction working solution and standard solutions should be stored protected from light.

9. Operating steps

1. Standard well: add 50 µL of standard solution with different concentrations into the wells. Sample well: add 50 µL of sample into the wells. Control well: add 50 µL of sample into the wells.
2. Add 10 µL of inhibitor into control wells.

3. Add 40 µL of reaction working solution into each well.
4. Mix fully with microplate reader for 5 s and incubate at 37°C for 10 min.
5. Add 10 µL of inhibitor into sample wells and standard wells immediately after incubation.
6. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm. The fluorescence values of the control and sample wells are respectively F_1, F_2, then ΔF = F_2 - F_1.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absolute fluorescence value.

3. Plot the standard curve by using absolute fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Definition: The amount of enzyme in 1 L of serum or plasma that catalyzes the production of 1 µmol resorufin per minute at 37°C is defined as 1 unit.

MPO Peroxidation activity (U/L) = (ΔF - b) ÷ a ÷ T × f

2. Tissue sample:

Definition: The amount of enzyme in 1 g of wet weight tissue that catalyzes the production of 1 µmol resorufin per minute at 37°C is defined as 1 unit.

MPO Peroxidation activity (U/g tissue wet weight) = (ΔF - b) ÷ a ÷ T × f ÷ (m/V) × 1000*

[Note]

ΔF: The absolute fluorescence value of sample, $F_2 - F_1$.

T: The reaction time, 10 min.

f: Dilution factor of sample before tested.

m: Wet weight of sample, g.

V: The volume of buffer solution.

1000*: 1U = 1000 mU

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.01	0.30	1.00
%CV	1.2	0.9	0.9

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

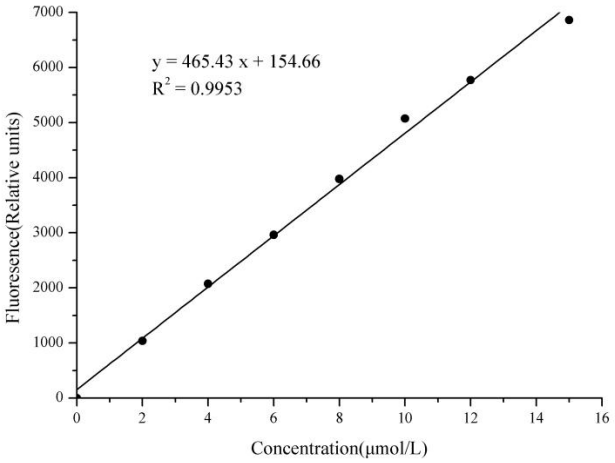
Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.01	0.30	1.00
%CV	5.2	4.8	6.2

Sensitivity

The analytical sensitivity of the assay is 0.001 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve

Concentration (μmol/L)	0	2	4	6	8	10	12	15
Fluorescence value	28	1055	2096	3079	4118	4973	5680	6717
	28	1079	2110	2904	3893	5233	5923	7063
Average fluorescence value	28	1067	2103	2992	4006	5103	5802	6890
Absolute fluorescent value	0	1039	2075	2964	3978	5075	5774	6862

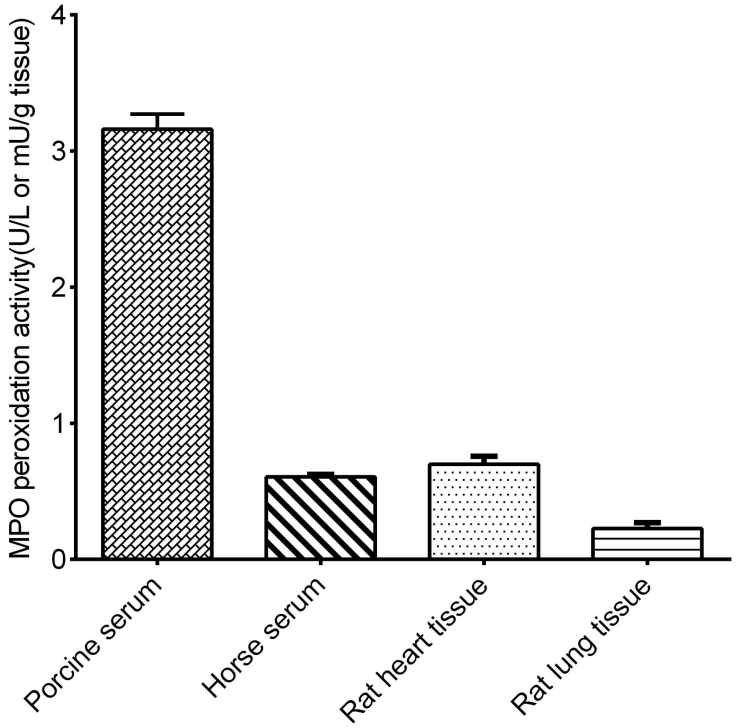
12. Appendix II Example Analysis

Example analysis:

For rabbit serum, add 50 μL of rabbit serum diluted 2-fold into corresponding wells, and carry out the assay according to the operation table. The results are as follows: standard curve: y = 466.97x + 74.669, the average fluorescence value of the sample is 4587 (F_2), the average fluorescence value of the control is 612 (F_1), then, $\Delta F = F_2 - F_1 = 3975$, and the calculation result is:

MPO Peroxidation activity (U/L) = (3975 - 74.669) ÷ 466.97 ÷ 10 × 2 = 1.67 U/L

Detect porcine serum (dilute 2-fold), horse serum (dilute 2-fold), 10% rat heart tissue homogenate and 10% rat lung tissue homogenate according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality

of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.