Technical Manual

Myeloperoxidase (MPO) Peroxidation Activity Fluorometric Assay Kit

- Catalogue Code: MAES0004
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Fluorimetric method

Specification:

96T

Range:

0.001 - 1.26 U/L

Sensitivity:

0.001 U/L

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Myeloperoxidase is a heme-containing cationic glycoprotein that belongs to the heme peroxidase family in mammals. MPO is a dimer formed by polymerization of two subunits. Each subunit contains a heavy chain and a light chain. MPO is abundant in the azuropathic granules of polymorphonuclear leukocytes (PMNLs) and a small number in monocytes and macrophages. Studies have shown that MPO plays an important role in the generation of oxidants and host defense in neutrophils and is closely related to the pathogenesis of many diseases, including cardiovascular disease, lung injury and cancer.

3. Intended Use

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

4. Detection Principle

Under the catalysis of peroxidase, hydrogen peroxide can oxidize the non-fluorescent probe into the 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.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	60 mL × 1 vial	-20°C, 6 months
Probe	0.25 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Substrate	0.25 mL × 1 vial	-20°C, 6 months
Inhibitor	1.2 mL × 1 vial	-20°C, 6 months
Resorufin Standard (25 µmol/L)	1.5 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Black Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Water bath
- Fluorescence microplate reader (Ex/Em=535 nm/587 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

1. After incubation add inhibitor to the sample and standard wells immediately.

2. The prepared reaction working solution and standard solutions should be stored with avoid direct sunlight.

7. Reagent Preparation

1. Bring all the reagents to room temperature before use.

2. The buffer solution is preheated at 37°C for 20 min, and can be used only after it is completely clarified.

3. Preparation of **reaction working solution:** Mix buffer solution, probe and substrate at a ratio of 36:2:2 fully. Prepare the fresh needed amount solution before use and store it with avoid direct sunlight.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of buffer solution (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.001 - 1.26 U/L).

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

Sample Type:	Dilution Factor
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.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: Ex/Em=535 nm/587 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'- S40', control wells.

10. Operation Steps

The preparation of standard curve

Dilute Resorufin (25 μmol/L) standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 15, 12, 10, 8, 6, 4, 2, 0 μmol/L.

The measurement of samples

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℃ 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 well are respectively F_1, F_2, then $\Delta F = F_2 - F_1$.

Operation Table

	Standard well	Sample well	Control well
Standard solution with different concentrations (μL)	50		
Samples (μL)		50	50
Inhibitor (μL)			10
Reaction working solution (μL)	40	40	40
Mix fully with microplate reader for 5 s and incubate at 37°C for 10 min			
Inhibitor (μL)	10	10	
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 well are respectively F_1, F_2, then $\Delta F = F_2 - F_1$.			

Email: info@assaygenie.com **Web:** www.assaygenie.com

11. Calculations

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample. The standard curve is: y= ax + b.

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

$$\text{MPO Peroxidation activity (U/L)} = (\triangle F - b) \div a \div T \times f$$

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

$$\text{MPO Peroxidation activity (U/g tissue wet weight)} = (\triangle F - b) \div a \div T \times f \div \frac{m}{V} \times 1000^{*}$$

y: The absolute fluorescence value of standard, $F_{Standard} - F_{Blank}$ (F_{Blank} is the F value when the standard concentration is 0)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

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

Email: info@assaygenie.com **Web:** www.assaygenie.com

12. Performance Characteristics

Detection Range	0.001 - 1.26 U/L
Sensitivity	0.001 U/L
Average inter-assay CV (%)	5.4
Average intra-assay CV (%)	1.0

Analysis

For Rabbit serum, add 50 µL of rabbit serum diluted for 2 times into corresponding wells, carry out the assay according to the operation table.

The results are as follows:

y = 466.97 x + 74.669, the average fluorescent value of the sample is 4587 (F_2), the average fluorescent value of the control is 612 (F_1), then, $\Delta F = F_2 - F_1$=3975, and the calculation result is:

$$\text{MPO Peroxidation activity (U/L)} = (3975 - 74.669) \div 466.97 \div 10 \times 2$$

$$= 1.67 \text{ U/L}$$

Email: info@assaygenie.com **Web:** www.assaygenie.com

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

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.

Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com