Myeloperoxidase (MPO) Peroxidation Activity Fluorometric Assay Kit

Catalog No: E-BC-F013

Method: Fluorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.001 U/L

Detection range: 0.001 - 1.26 U/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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.

Hydrogen peroxide + Substrate $\xrightarrow{\text{Peroxidation}}$ Fluorescence value F2 (Ex/Em=535 nm/587 nm)

Hydrogen peroxide + Substrate + MPO inhibitor $\xrightarrow{\text{Peroxidation}}$ Fluorescence value F1

Fluorescence value of myeloperoxidase peroxidation activity
= Fluorescence value F2 - Fluorescence value F1

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	-20℃ , 6 months
Reagent 2	Probe	0.25 mL × 1 vial	-20℃ , 6 months, shading light
Reagent 3	Substrate	0.25 mL × 1 vial	-20℃ , 6 months
Reagent 4	Inhibitor	1.2 mL × 1 vial	-20℃ , 6 months
Reagent 5	25 μmol/L Resorufin Standard	1.5 mL × 1 vial	-20℃ , 6 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

▲Materials prepared by users

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Micropipette, Vortex mixer, Water bath

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

▲The key points of the assay

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

Pre-assay preparation

▲Reagent preparation

1. Bring all the reagents to room temperature before use.
2. The reagent 1 is preheated at 37℃ for 20 min, and can be used only after it is completely clarified.
3. Preparation of reaction working solution:
   Mix reagent 1, reagent 2 and reagent 3 at a ratio of 36:2:2 fully. Prepare the fresh needed amount solution before use and store it with shading light.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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 reagent 1.

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	Ex/Em=535 nm/587 nm

Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲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', sample wells.

▲Operating steps

1. The preparation of standard curve

   Dilute 25 μmol/L resorufin standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 15, 12, 10, 8, 6, 4, 2, 0 μmol/L.

2. 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 reagent 4 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 reagent 4 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 F1, F2, then ΔF = F2 - F1.

▲Operation table

	Standard well	Sample well	Control well
Standard solution with different concentrations (μL)	50		
Samples (μL)		50	50
Reagent 4 (μL)			10
Reaction working solution (μL)	40	40	40
Mix fully with microplate reader for 5 s and incubate at 37℃ for 10 min			
Reagent 4 (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 F1, F2, then ΔF = F2 - F1.			

▲Calculation

Plot the standard curve by using fluorescent value 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 fluorescent value of sample.
The standard curve is: y= ax + b.

1. Serum (plasma) sample:

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

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

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℃ is defined as 1 unit.

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

Note:

y: $F_{Standard} - F_{Blank}$ (F_{Blank} is the fluorescence 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 reagent 1.

*: 1U = 1000 mU

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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

▲Example analysis

For rabbit serum, add 50 μL of rabbit serum diluted for 2 times into corresponding wells, and carry the assay according to the operation table. The results are as follows: standard curve: 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:

$$\text{MPO Peroxidation activity(U/L)} = \frac{(3975 - 74.669)}{466.97} \div 10 \times 2 = 1.67 \text{ U/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4℃ for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection.

▲ Plasma

The fresh blood was added into the test tube containing anticoagulant and mixed upside down. Centrifuge the sample at 4℃ for 10 min at 700~1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection.

▲ Tissue sample

Accurately weigh the tissue sample, add reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

Note:

1. Homogenized medium: Reagent 1.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

▲ Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.