Myeloperoxidase (MPO) Activity Assay Kit

Catalog No: E-BC-K074-M

Method: Colorimetric method

Specification: 96T (Can detect 48 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 19.42 U/L

Detection range: 19.42-893.31 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) activity in serum, plasma, milk, animal tissue.

▲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

Myeloperoxidase reduces hydrogen peroxide to a complex. The complex react with o-dianisidine (as hydrogen donor) to produce a yellow product which has a maximum absorption peak at 460 nm. The activity of MPO can be calculated indirectly by measuring the OD value at 460nm.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	2-8℃ , 6 months
Reagent 2	Powder A	Powder × 2 vials	2-8℃ , 6 months
Reagent 3	Powder B	Powder × 2 vials	2-8℃ , 6 months
Reagent 4	Saline Solution	6 mL × 1 vial	2-8℃ , 6 months
Reagent 5	Clarificant	1.2 mL × 2 vials	2-8℃ , 6 months
Reagent 6	Powder C	Powder × 2 vials	2-8℃ , 6 months, shading light
Reagent 7	Substrate	0.1 mL × 1vial	2-8℃ , 6 months
Reagent 8	Acid Reagent	1 mL × 1 vial	2-8℃ , 6 months
	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

Microplate reader (460 nm), Tubes, Micropipette, Vortex mixer, 37°C /60°C 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. The supernatant must be clarified after centrifugation during the operation step.
2. If 5% tissue homogenate is used for the experiment, the OD value is very low. 10% tissue homogenate can be used for the experiment, that is, the reagent 2 application solution is added according to the proportion of tissue weight (g) : volume (mL)=1:9, and the homogenate time is increased to make the tissue grind as far as possible.
3. Chelating agents such as EDTA should not be added to the sample.

Pre-assay preparation

▲Reagent preparation

1. Preparation of Reagent 1 application solution:
   Prepare the required amount according to the ratio of reagent 1: double distilled water =1:9. The prepared solution can be stored at 2-8°C for 1 month.
2. Preparation of Reagent 2 application solution:
   Dissolve a vial of powder A with 60 mL reagent 1 application solution before use, or heat at 37°C to dissolve, store at 2-8°C for 2 weeks.
3. Preparation of Reagent 3 application solution:
   Add 1 vial of powder B into 3 mL reagent 4 to dissolve completely before use. The prepared solution can be store at 2-8°C for 2 weeks.
4. Preparation of chromogenic agent:
   Dissolve a vial of powder C with 12.5 mL reagent 1 application solution fully, then add 12.5 μL reagent 7, mix fully and store at 2-8°C with shading light.
5. If reagent 5 freeze in cold condition, shake in 37°C water bath to dissolve fully (transparent) before use.

▲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 (19.42-893.31 U/L).

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

Sample type	Dilution factor
Human serum	1
Human plasma	1
Human milk	1
Cell culture supernatant	1
Rat serum	1
Rat plasma	1
5% Rat kidney tissue homogenate	1
5% Rat spleen tissue homogenate	1

Note: The diluent is reagent 2 application solution.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	460 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	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'
H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'	S48	S48'

Note: S1-S48, sample wells; S1'-S48', control wells.

▲Operating steps

Sample pretreatment

Tissue sample: Take 90 μL of tissue homogenate and add 10 μL of reagent 3 application solution, mix fully and incubate at 37°C for 15 min.

Serum (plasma): Take 45 μL of sample and add 45 μL of reagent 2 application solution, mix fully, then add 10 μL of reagent 3 application solution and incubate at 37°C for 15 min.

The measurement of samples

1) Control tube: Add 350 μL of double distilled water, 20 μL of sample, 20 μL of reagent 5 into 1.5 mL EP tubes.
   Sample tube: Add 20 μL of sample, 20 μL of Reagent 5, 350 μL of chromogenic agent into 1.5 mL EP tubes.
2) Oscillate fully with a vortex mixer and incubate for 30 min at 37°C .
3) Add 5 μL of reagent 8, oscillate fully with a vortex mixer and incubate for 10 min at 60°C .
4) Centrifuge the tubes at 3000 g for 10 min and take 300 μL of supernatant for measuring the OD value. Measure the OD value of each well at 460 nm with microplate reader.

▲Operation table

	Control tube	Sample tube
Double distilled water (μL)	350	
Sample (μL)	20	20
Reagent 5 (μL)	20	20
Chromogenic agent (μL)		350
Mix thoroughly, incubate at 37°C water bath for 30 min.		
Reagent 8 (μL)	5	5
Mix thoroughly and incubate at 60°C water bath for 10 min. Centrifuge the tubes at 3000 g for 10 min and take 300 μL of supernatant. Measure the OD value of each well at 460 nm with microplate reader.		

Note: if the reaction solution appears solidification state, the OD value will increase, it is recommended to incubate the reaction solution at 37℃ and measure the OD value after the solidification state is disappeared

▲Calculation

Serum (plasma) and other liquid sample:

Definition: The amount of MPO in 1 L of sample that catalyze decomposition of 1 μmol H_2O_2 at 37 °C for 30 min is defined as 1 unit.

$$\text{MPO activity(U/L)}=\frac{\Delta A}{11.3^{*}\times b}\times V_{Total}\div\left(\frac{V_{Sample}}{V_1}\times V_2\right)\times 1000\times f$$

$$=\frac{0.175\times 1000\times\Delta A}{V_{Sample}}\times f$$

Tissue sample:

Definition: The amount of MPO in 1 g wet weight of tissue that catalyze decomposition of 1 μmol H_2O_2 at 37 °C for 30 min is defined as 1 unit.

$$\text{MPO activity(U/g wet weight)}=\frac{\Delta A}{11.3^{*}\times b}\times V_{Total}\div\left(\frac{m}{V_3}\times V_2\times 0.9\right)$$

$$=\frac{1.942\times V_3\times\Delta A}{m}$$

Note:

ΔA: OD_{sample}-$OD_{control}$

11.3*: constant

b: optical path of the quartz cuvette, 1 cm.

V_{Total}: the total volume of reaction system, 0.395 mL.

V_{Sample}: the volume of sample added in sample pretreatment step for serum (plasma) and milk sample, 0.045 mL

V_1: the total volume in sample pretreatment step, 0.045+0.045+0.01=0.1 mL or 0.09+0.01=0.1 mL.

V_2: the volume of sample added to reaction system, 0.02 mL.

V_3: the volume of reagent 2 application solution added into tissue sample in sample preparation step;

1000: 1 L=1000 mL;

m: wet weight of sample, g;

0.9: the ratio of sample volume and total volume in sample pretreatment step, 0.09 mL/0.1 mL=0.9.

f: the dilution factor of sample before tested.

▲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

Appendix I Performance characteristics			
Detection range	19.42-893.31 U/L	Average intra-assay CV (%)	5.4
Sensitivity	19.42 U/L	Average inter-assay CV (%)	7.3
Average recovery rate (%)	104		

▲Example analysis

For human serum, take 45 μL of human serum, and carry the assay according to the operation table. The results are as follows: the average OD value of the sample is 0.116, the average OD value of the control is 0.061

The calculation result is:

$$\text{MPO activity(U/L)} = \frac{0.116-0.061}{0.045} \times 0.175 \times 1000 = 213.89 \text{ U/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum/ Plasma sample: After sample pretreatment detect directly

▲Milk sample:

Collect fresh milk sample, centrifuge the sample at 4°C for 10 min at 10000 g, discard the upper white liquid, take the middle layer liquid and preserve the sample on ice for detection.

▲5% tissue homogenate sample:

Accurately weigh the tissue sample, add Reagent 2 application solution according to the ratio of Weight (g): Volume (mL) =1:19. Mechanical homogenate the sample in ice water bath. Don't centrifuge, preserve the sample on ice for detection.

▲Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the detection, 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.