PRODUCT INFORMATION & MANUAL

Myeloperoxidase/MPO Activity Assay Kit (Colorimetric)

NBP3-24523

For research use only.
Not for diagnostic or therapeutic procedures.

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Myeloperoxidase/MPO Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24523

Method: Colorimetric method

Specification: 100 Assays (Can detect 50 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 16.95 U/L

Detection range: 16.95-3349 U/L

Average intra-assay CV (%): 4.5

Average inter-assay CV (%): 9.8

Average recovery rate (%): 104

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ 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, cells 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

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	36 mL× 1 vial	2-8℃ , 12 months
Reagent 2	Powder A	2 vials	2-8℃ , 12 months
Reagent 3	Powder B	2 vials	2-8℃ , 12 months
Reagent 4	Saline Solution	10 mL× 2 vials	2-8℃ , 12 months
Reagent 5	Clarificant	24 mL× 1 vial	2-8℃ , 12 months
Reagent 6	Powder C	2 vials	2-8℃ , 12 months, shading light
Reagent 7	Substrate	0.3 mL× 1 vial	2-8℃ , 12 months
Reagent 8	Acid Reagent	6 mL× 1 vial	2-8℃ , 12 months

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.

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

▲Materials prepared by users

Instruments

Spectrophotometer (460nm), Vortex mixer, Micropipettor, Water bath, Incubator, Centrifuge

Reagents

Double distilled water or deionized 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 point of the assay

The supernatant after centrifugation must be clarified.

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℃ for 1 month.
2. Preparation of reagent 2 application solution
   Dissolve a vial of powder A with 60 mLof reagent 1 application solution before use, or heat at 37℃ to dissolve, store at 2-8℃ for 2 weeks. If there is precipitation, it can be heated and dissolved at 37℃ before use.
3. Preparation of reagent 3 application solution
   Add 1 vial of powder B into 1 vial of reagent 4 to dissolve completely before use. The prepared solution can be store at 2-8℃ for 2 weeks.
4. Reagent 5 will freeze in cold condition, shake in 37℃ water bath to dissolve fully (transparent) before use.
5. Preparation of chromogenic agent
   Dissolve a vial of powder C with 100 mL of reagent 1 application solution fully, then add 0.1 mL of reagent 7, mix fully and store at 2-8℃ with shading light.

▲Sample preparation

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

Sample requirements

The sample should not contain chelating agents such as EDTA.

▲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 (4.9-196.7 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
Rat serum	1
5% Mouse brain tissue homogenization	1
5% Mouse heart tissue homogenization	1
Human milk	1

Note: The diluent is reagent 2 application solution.

Assay protocol

▲Detailed operation steps

Sample pretreatment

1) For serum (plasma) and milk sample:
   Take 0.45 mL of sample and add 0.45 of reagent 2 application solution, mix fully, then add 0.1 of reagent 3 application solution and incubate at 37℃ for 15 min.
2) For tissue and cells sample:
   Take 0.9 mL of sample and add 0.1 of reagent 3 application solution, mix fully and incubate at 37℃ for 15 min.

The measurement of samples

1) Control tube: Add 3 mL of double distilled water, 0.2 mL of sample, 0.2 mL of reagent 5 into 5 mL EP tubes.
   Sample tube: Add 0.2 mL of sample, 0.2 mL of reagent 5, 3 mL of chromogenic agent into 5 mL EP tubes.
2) Oscillate fully with a vortex mixer and incubate for 30 min at 37℃ .
3) Add 0.05 mL of reagent 8, oscillate fully with a vortex mixer and incubate for 10 min at 60℃ water bath.
4) Centrifuge the tubes at 2325 g for 10 min and take the supernatant for measuring the OD value.
5) Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 460 nm with 1.0 cm optical path cuvette immediately.

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.

▲Summary operation table

	Control tube	Sample tube
Double distilled water (mL)	3	
Sample (mL)	0.2	0.2
Reagent 5 (mL)	0.2	0.2
Chromogenic agent (mL)		3
Mix thoroughly, incubate at 37℃ water bath for 30 min.		
Reagent 8 (mL)	0.05	0.05
Mix thoroughly and incubate at 60℃ water bath for 10 min. Centrifuge the tubes at 2325 g for 10 min and take the supernatant. Set the spectrophotometer to zero and measure the OD value of each tube.		

▲Calculation

1. For serum (plasma) and milk sample

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

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

$$= \frac{1.526 \times 1000 \times \Delta A}{V_{Sample}} \times f$$

2. For 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 ℃ for 30 mnin is defined as 1 unit.

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

$$= \frac{1.696 \times V_3 \times \Delta A}{m}$$

3. For cells sample

Definition:The amount of MPO in 1×10^6 cells that catalyze decomposition of 1 μmol H_2O_2 at 37℃ for 30 mnin is defined as 1 unit.

$$\text{MPO activity (U/}10^6\text{)} = \frac{\Delta A}{11.3^{*} \times b} \times V_{Total} \div \left(\frac{N}{V_3} \times V_2 \times 0.9\right)$$

$$= \frac{1.696 \times V_3 \times \Delta A}{N}$$

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, 3.45 mL.

V_{Sample}: the volume of sample added in sample pretreatment step for serum (plasma) and milk sample, 0.45 mL

V_1: the total volume in sample pretreatment step, 0.45+0.45+0.1=1 mL or 0.9+0.1=1 mL.

V_2: the volume of sample added to reaction system, 0.2 mL.

V_3: the volume of reagent 2 application solution added into tissue or cell sample in sample preparation step (Appendix II);

1000: 1 L=1000 mL;

m: wet weight of sample, g;

N: the number of cells;

0.9: the ratio of sample volume and total volume in sample pretreatment step, 0.9 mL/1 mL=0.9.

f: the dilution factor of sample before tested.

Appendix I Data

▲Example analysis

Take 0.45 mL 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 0.089, the average OD value of the control is 0.007, and the calculation result is:

$$\text{MPO activity (U/L)} = \frac{0.089-0.007}{0.45} \times 1.526 \times 1000 \times 1 = 278.07 \text{ U/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . 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℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4℃ . 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℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenization medium (2-8℃) (mL): the weight of the tissue (g) =19:1, take the tissue homogenate to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲ Milk sample

Collect the milk sample, centrifuge at 10000 g for 10 min at 4℃ and collect middle layer liquid to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Cells

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Then take the cell homogenate and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: reagent 2 application solution.

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.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2s/time, interval for 3 s, the total time is 5 min).

Appendix III References

1. Prokopowicz Z, Marcinkiewicz J, Katz D R, et al. Neutrophil Myeloperoxidase: Soldier and Statesman[J]. Archivum Immunologiae Et Therapiae Experimentalis, 2012, 60(1): 43-54.
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