（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K074-M

Specification：48T(24 samples)/ 96T(48 samples)

Measuring instrument：Microplate reader（460 nm）

Detection range：19.42-893.31 U/L

Elabscience®Myeloperoxidase (MPO) Activity Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

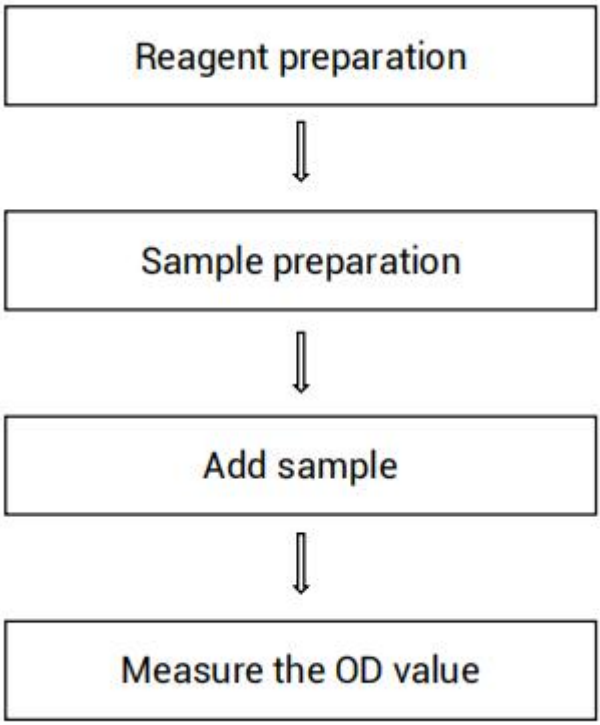
Email: techsupport@elabscience.com

Website: www.elabscience.com

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

Table of contents

Assay summary



Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL ×1 vial	20 mL × 1 vial	2-8℃, 12 months
Reagent 2	Powder A	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months
Reagent 3	Powder B	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months
Reagent 4	Saline Solution	3 mL × 1 vial	6 mL × 1 vial	2-8℃, 12 months
Reagent 5	Clarificant	1.2 mL ×1 vial	1.2 mL × 2 vials	2-8℃, 12 months
Reagent 6	Powder C	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months, shading light
Reagent 7	Substrate	0.1 mL × 1vial	0.1 mL × 1vial	2-8℃, 12 months
Reagent 8	Acid Reagent	1 mL × 1 vial	1 mL × 1 vial	2-8℃, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (460 nm), Tubes, Micropipette, Vortex mixer, 37℃/60℃ Water bath

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of buffer application solution：
Dilute 6 mL of buffer solution with 54 mL of double distilled water, mix well. Store at 2-8°C for 1 month.

③ The preparation of powder A application solution：
Dissolve one vial of powder A with 60 mL of buffer application solution, or heat at 37℃ to dissolve. Store at 2-8°C for 2 weeks.

④ The preparation of powder B application solution：
Dissolve one vial of powder B with 3 mL of saline solution, mix well to dissolve. Store at 2-8°C for 2 weeks.

⑤ The preparation of chromogenic agent：
Dissolve one vial of powder C with 12.5 mL of buffer application solution, mix well to dissolve. And add 12.5 μL of substrate, mix well to dissolve. Store at 2-8°C protected from light.

⑥ If clarificant freeze in cold condition, shake in 37℃ water bath to dissolve fully (transparent) before use.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ 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 powder A application solution with a dounce homogenizer at 4℃.

④ Collect sample and keep it on ice for detection.

② Dilution of sample

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
10% Rat kidney tissue homogenate	1
10% Rat spleen tissue homogenate	1

Note: The diluent is powder A application solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① The supernatant must be clarified after centrifugation during the operation step.

② If 10% tissue homogenate is used for the experiment, the OD value is very low. 20% 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)=1:4, 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.

Operating steps

Sample pretreatment:

Tissue sample: Take 90 μL of tissue homogenate and add 10 μL of powder B application solution, mix fully and incubate at 37℃ for 15 min.

Serum (plasma): Take 45 μL of sample and add 45 μL of powder A application solution, mix fully, then add 10 μL of powder B application solution and incubate at 37℃ for 15 min.

The measurement of samples:

① Control tube: Add 350 μL of double distilled water, 20 μL of sample, 20 μL of clarificant into 1.5 mL EP tubes.

Sample tube: Add 20 μL of sample, 20 μL of clarificant, 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℃.

③ Add 5 μL of acid reagent, oscillate fully with a vortex mixer and incubate for 10 min at 60℃.

④ Centrifuge the tubes at 3000×g for 10 min and take 300 μL of supernatant for measuring the OD value.

⑤ Measure the OD value at 460 nm with microplate reader.

Calculation

The sample:

1. Serum (plasma) sample:

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

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

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

2. 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 min is defined as 1 unit

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

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

[Note]

ΔA: OD_{sample}-$OD_{control}$.

11.3*: constant.

b: The 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 powder A application solution added into tissue sample in sample preparation step.

1000: 1 L=1000 mL.

m: The 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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	36.50	264.50	579.60
%CV	5.8	5.4	5.0

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	36.50	264.50	579.60
%CV	7.2	7.5	7.2

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 104%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	95.6	342.5	678.2
Observed Conc. (U/L)	95.6	363.1	718.9
recovery rate(%)	100	106	106

Sensitivity

The analytical sensitivity of the assay is 19.42 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.

Appendix Π Example Analysis

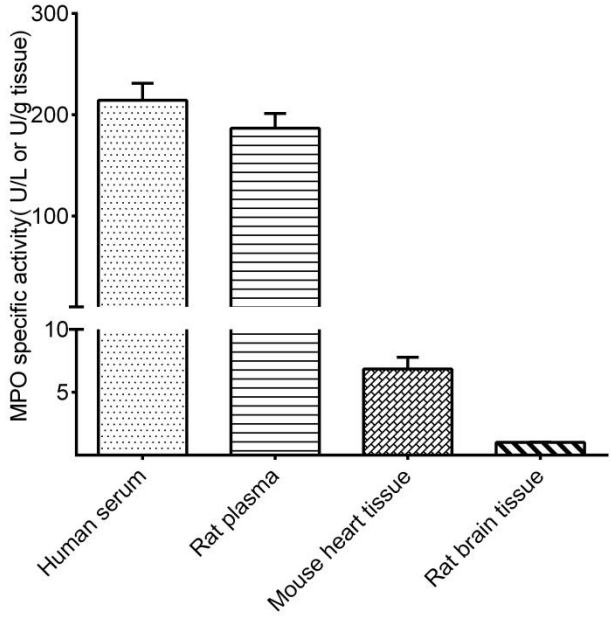
Example analysis：

For human serum, take 45 μL of human serum, and carry the assay according to the operation steps. 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, and the calculation result is:

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

Detect human serum (45 μL), rat plasma (45 μL), 5% mouse heart tissue homogenate tissue (90 μL), 5% rat brain tissue homogenate tissue (90 μL), according to the protocol, the result is as follows：



Appendix III Publications

1. CXCL5 activates CXCR2 in nociceptive sensory neurons to drive joint pain and inflammation in experimental gouty arthritis[J].Nature communications, 15(1):3263.DOI:10.1038/s41467-024-47640-7.
2. Liu Z , Liu B , Feng Y ,et al.Dual-Targeted Self-Adjuvant Heterocyclic Lipidoid@Polyester Hybrid Nanovaccines for Boosting Cancer Immunotherapy[J].ACS Nano, 2024, 18(24):19.DOI:10.1021/acsnano.4c00392.
3. Liang L , Peng W , Qin A ,et al.Intracellularly Synthesized Artificial Exosome Treats Acute Lung Injury[J].ACS Nano, 2024, 18(32):15.DOI:10.1021/acsnano.4c01900.
4. Wang D , Wang K , Liu Q ,et al.A Novel Drug Candidate for Sepsis Targeting Heparanase by Inhibiting Cytokine Storm (Adv. Sci. 29/2024)[J].Advanced Science, 2024, 11(29).DOI:10.1002/advs.202470175.
5. Zhan Y , Lou H , Shou R ,et al.Maternal exposure to E 551 during pregnancy leads to genome-wide DNA methylation changes and metabolic disorders in the livers of pregnant mice and their fetuses[J].Journal of Hazardous Materials, 2024(Mar.5):465.DOI:10.1016/j.jhazmat.2023.133233.
6. Hu C , Yuan X , Zhao R ,et al.Scale - Up Preparation of Manganese - Iron Prussian Blue Nanozymes as Potent Oral Nanomedicines for Acute Ulcerative Colitis[J].Advanced Healthcare Materials, 2024, 13(16).DOI:10.1002/adhm.202400083.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.