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

Catalog No：E-BC-K074-S

Specification：50 assays(25 samples)/100 assays(50 samples)

Measuring instrument：Spectrophotometer（460 nm）

Detection range：16.95-3349 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.

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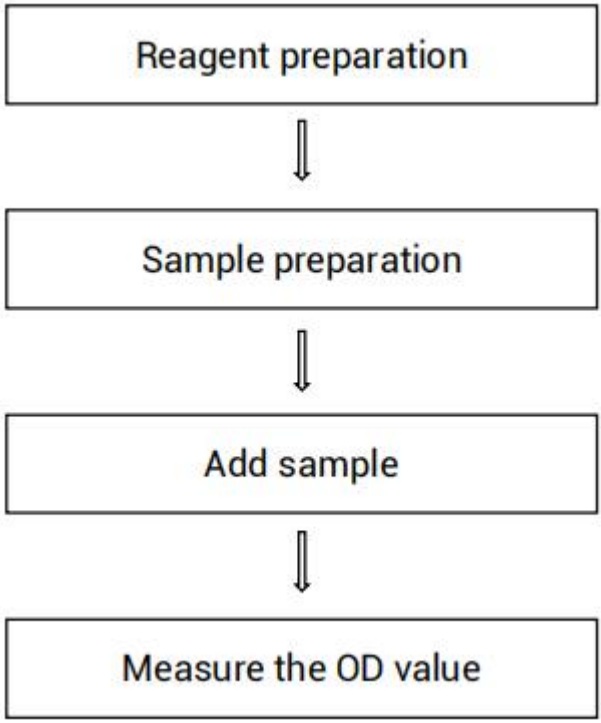
Assay summary



Intended use

This kit can be used to detect myeloperoxidase (MPO) activity in serum, plasma, milk, animal tissue, cells and other 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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Buffer Solution	18 mL ×1 vial	36 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	10 mL × 1 vial	10 mL × 2 vials	2-8℃, 12 months
Reagent 5	Clarificant	12 mL ×1 vial	24 mL × 1 vial	2-8℃, 12 months
Reagent 6	Powder C	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months, shading light
Reagent 7	Substrate	0.15 mL × 1 vial	0.3 mL × 1 vial	2-8℃, 12 months
Reagent 8	Acid Reagent	3 mL × 1 vial	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water or deionized 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 one vial 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 100 mL of buffer application solution, mix well to dissolve. And add 100 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 380 μL powder A application solution with a dounce homogenizer at 4℃.

④ Collect sample and keep it on ice for detection.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 2×10^6 cells in 400 μL powder A application solution with a ultrasonic cell disruptor 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
Rat serum	1
5% Mouse brain tissue homogenization	1
5% Mouse heart tissue homogenization	1
Human milk	1
Human serum	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

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

Operating steps

Sample pretreatment:

Tissue and cell sample: Take 900 μL of tissue or cell homogenate and add 100 μL of powder B application solution, mix fully and incubate at 37℃ for 15 min.

Serum (plasma): Take 450 μL of sample and add 450 μL of powder A application solution, mix fully, then add 100 μL of powder B application solution 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 clarificant into 5 mL EP tubes.
Sample tube: Add 0.2 mL of sample, 0.2 mL of clarificant, 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 acid reagent, oscillate fully with a vortex mixer and incubate for 10 min at 60℃.

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.

Calculation

The sample:

1. 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 min is defined as 1 unit.

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

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

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

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

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

3. 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 min is defined as 1 unit.

$$\text{MPO activity (U/10}_6\text{)} = \frac{\Delta A}{11.3^* \times b} \times V_{Total} \div \left(\frac{N}{V_3} \times V_2 \times 0.9\right) \times f$$

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

[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, 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.

1000: 1 L=1000 mL.

m: The 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 teste.

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)	52.50	567.80	1126.00
%CV	4.8	4.4	4.3

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)	52.50	567.80	1126.00
%CV	10.2	9.4	9.8

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)	684.5	1354	2686.5
Observed Conc. (U/L)	711.9	1435.2	2740.2
recovery rate(%)	104	106	102

Sensitivity

The analytical sensitivity of the assay is 16.95 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

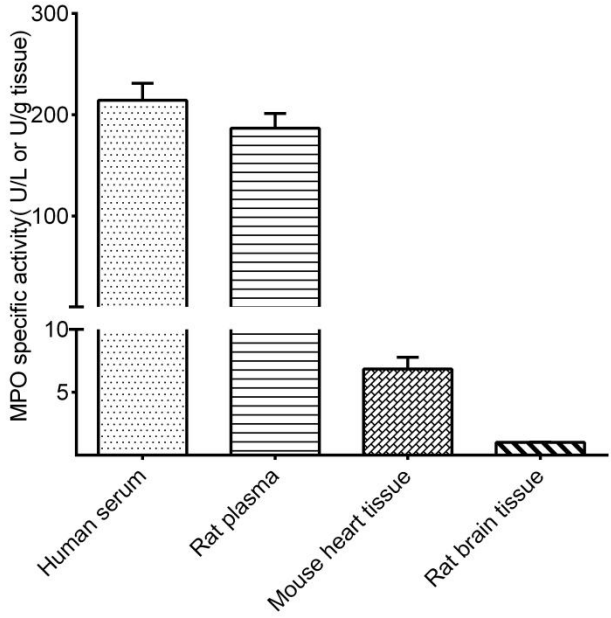
Example analysis：

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

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

Detect human serum, rat plasma, 5% rat heart tissue homogenate and 5% mouse brain tissue homogenate according to the protocol, the result is as follows：



Appendix III Publications

1. 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.
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. Hu M , Du H , Xu Y ,et al.Gentiopicroside Ameliorates Sepsis - Induced Acute Lung Injury via Inhibiting Inflammatory Response[J].Canadian Respiratory Journal, 2024, 2024.DOI:10.1155/2024/1068326.
4. Yin C , Lyu Q , Dong Z ,et al.Well-defined alginate oligosaccharides ameliorate joint pain and inflammation in a mouse model of gouty arthritis[J].Theranostics, 2024, 14(8).DOI:10.7150/thno.95611.
5. Chen H , Pan L , Zhang C ,et al.Gastroretentive Raft Forming System for Enhancing Therapeutic Effect of Drug - Loaded Hollow Mesoporous Silica on Gastric Ulcers[J].Advanced Healthcare Materials, 2024, 13(22).DOI:10.1002/adhm.202400566.

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.