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

Catalog No：E-BC-F013

Specification：96T(40 samples) / 500Assays(242 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.001 - 1.26 U/L

Elabscience®Myeloperoxidase (MPO) Peroxidation Activity Fluorometric 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 3

Intended use 4

Detection principle 4

Kit components & storage 5

Materials prepared by users 5

Reagent preparation 5

Sample preparation 7

The key points of the assay 8

Operating steps 8

Calculation 9

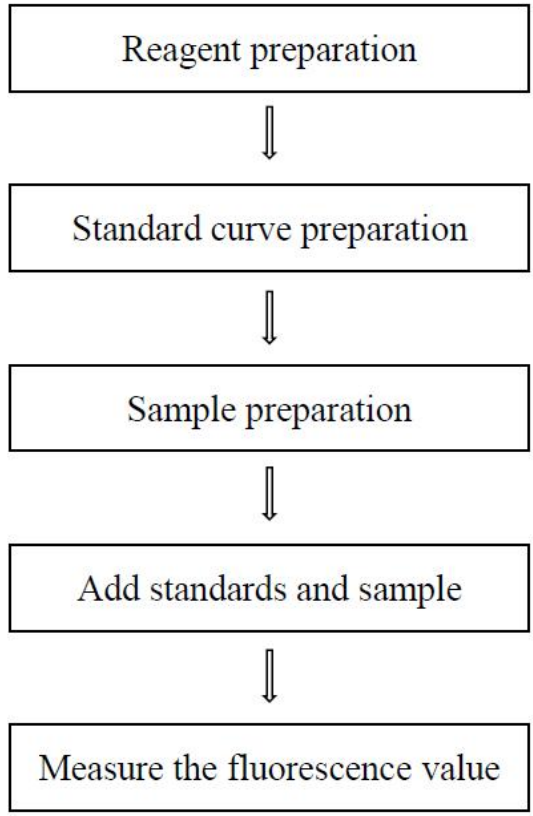
Appendix I Performance Characteristics 10

Appendix II Example Analysis 12

Appendix III Publications 13

Statement 14

Assay summary



Intended use

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

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 F_2 (Ex/Em=535 nm/587 nm)

Hydrogen peroxide + Substrate + MPO inhibitor $\xrightarrow{\text{Peroxidation}}$ Fluorescence value F_1

Fluorescence value of myeloperoxidase peroxidation activity = Fluorescence value F_2 - Fluorescence value F_1

Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500Assays)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	60 mL × 5 vials	-20℃, 12 months
Reagent 2	Probe	0.25 mL × 1 vial	1.25 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Substrate	0.25 mL × 1 vial	1.25 mL × 1 vial	-20℃, 12 months
Reagent 4	Inhibitor	1.2 mL × 1 vial	6 mL × 1 vial	-20℃, 12 months
Reagent 5	25 μmol/L Resorufin Standard	1.5 mL × 1 vial	7.5 mL × 1 vial	-20℃, 12 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The buffer solution is preheated at 37℃ for 20 min, and can be used only after it is completely clarified.

③ The preparation of reaction working solution：

For each well, prepare 40 μL of reaction working solution (mix well 36 μL of

buffer solution, 2 μL of probe and 2 μL of substrate). The reaction working solution should be prepared on spot and protected from light.

④ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**2**	**4**	**6**	**8**	**10**	**12**	**15**
25 μmol/L standard (μL)	0	20	40	60	80	100	120	150
Buffer solution (μL)	250	230	210	190	170	150	130	100

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 buffer solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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 buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

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.

Operating steps

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 inhibitor 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 inhibitor 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 F_1, F_2, then $\Delta F = F_2 - F_1$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

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.

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

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.

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

[Note]

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 buffer solution.

1000*: 1U = 1000 mU

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)	0.01	0.30	1.00
%CV	1.2	0.9	0.9

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.01	0.30	1.00
%CV	5.2	4.8	6.2

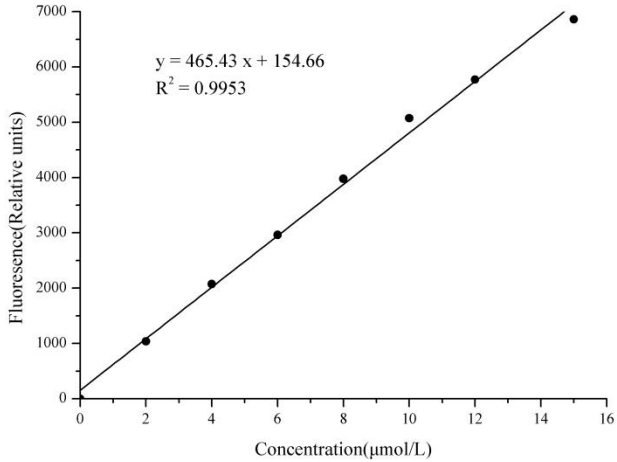
Sensitivity

The analytical sensitivity of the assay is 0.001 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.

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	0	2	4	6	8	10	12	15
Fluorescence value	28	1055	2096	3079	4118	4973	5680	6717
	28	1079	2110	2904	3893	5233	5923	7063
Average fluorescence value	28	1067	2103	2992	4006	5103	5802	6890
Absoluted fluorescent value	0	1039	2075	2964	3978	5075	5774	6862



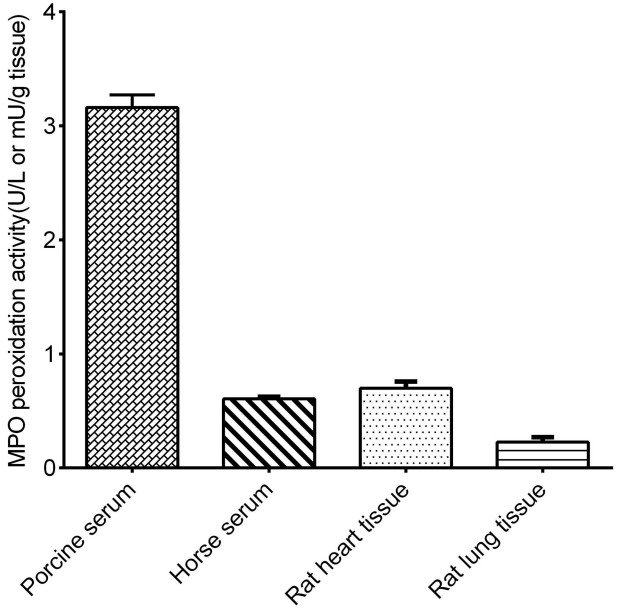
Appendix II Example Analysis

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 fluorescence value of the sample is 4587 (F_2), the average fluorescence value of the control is 612 (F_1), then, $\Delta F = F_2 - F_1$=3975, and the calculation result is:

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

Detect porcine serum (dilute for 2 times), horse serum (dilute for 2 times), 10% Rat heart tissue homogenate and 10% Rat lung tissue homogenate according to the protocol, the result is as follows:



Appendix III Publications

1. Wang J, Kang G, Lu H, et al. Novel bispecific nanobody mitigates experimental intestinal inflammation in mice by targeting TNF-α and IL-23p19 bioactivities[J]. Clinical and translational medicine, 2024, 14(3): e1636.
2. Yang Y, Chen Q, Liu Z, et al. Novel reduced heteropolyacid nanoparticles for effective treatment of drug-induced liver injury by manipulating reactive oxygen and nitrogen species and inflammatory signals[J]. Journal of Colloid and Interface Science, 2025, 678: 174-187.
3. Chen Y, Liu H, Tian Y, et al. Fexofenadine protects against lipopolysaccharide-induced acute lung injury by targeting cytosolic phospholipase A2[J]. International Immunopharmacology, 2023, 116: 109637.
4. Yu R, Zhou Q, Liu T, et al. Kaempferol relieves the DSS-induced chronic colitis in C57BL/6J mice, alleviates intestinal angiogenesis, and regulates colonic microflora structure[J]. Journal of Functional Foods, 2023, 107: 105646.
5. Chen Y, Liu H, Zhang Q, et al. Cinacalcet targets the neurokinin-1 receptor and inhibits PKCδ/ERK/P65 signaling to alleviate dextran sulfate sodium-induced colitis[J]. Frontiers in Pharmacology, 2021, 12: 735194.
6. Shaikh S A, Muthuraman A. Tocotrienol-Rich Fraction Ameliorates the Aluminium Chloride-Induced Neurovascular Dysfunction-Associated Vascular Dementia in Rats[J]. Pharmaceuticals, 2023, 16(6): 828.

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.