CheKine™ Micro Myeloperoxidase (MPO) Activity Assay Kit

Cat #: KTB1152 Size: 48 T/96 T

	Micro Myeloperoxidase (MPO) Activity Assay Kit		
REF	**Cat #:** KTB1152	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissue, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Myeloperoxidase (MPO) is a kind of leukocyte enzyme secreted by activated neutrophils and mononuclear macrophages, which mainly exists in the aniline-blue granules of neutrophils and mononuclear macrophages. When white blood cells are activated, MPO is released into phagocytic vesicles and plasma, inducing oxidative stress and tissue damage under specific conditions, and is a marker of systemic inflammation and oxidative stress. CheKine™ Micro Myeloperoxidase (MPO) Activity Assay Kit can be used to detect biological samples such as animal tissue, cells, plasma, serum or other liquid samples. In the kit, MPO catalyzes the decomposition of H_2O_2, and at the same time oxidizes o-dianiside to produce colored substances. There is a characteristic absorption peak at 460 nm, and the color depth is linear with MPO activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	100 mL	100 mL×2	4℃
Reagent Ⅱ	1	1×2	4℃, protected from light
Reagent Ⅲ	1	1	4℃, protected from light
Reagent Ⅳ	200 μL	400 μL	4℃, protected from light
Reagent Ⅴ	400 μL	800 μL	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 460 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Reagent Ⅱ: Prepared before use. After add 50 mL Reagent Ⅰ to each bottle to dissolve thoroughly. The prepared reagent can be stored at 4℃, protected from light for 2 weeks.

Working Reagent Ⅲ: Prepared before use. Add 15 mL Reagent Ⅰ for 48 T and 30 mL Reagent Ⅰ for 96 T to fully dissolve. The prepared reagent can be stored at 4℃, protected from light for 1 month.

Note: Working Reagent Ⅱ and Working Reagent Ⅲ is difficult to dissolve and can be heated at 37℃ and violently oscillated to promote dissolution, or treated with ultrasound to promote dissolution. Check whether there is powder precipitation before each use. If dissolving Working Reagent Ⅱ at high temperature, allow it to cool to room temperature before using.

Reagent Ⅳ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Working Reagent: For each well, prepare 270 μL Working Reagent. Mix Working Reagent Ⅲ and Reagent Ⅳ at a volume ratio of 999:1. Working Reagent is freshly prepared.

Reagent Ⅴ: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃, protected from light.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Working Reagent Ⅱ and homogenize or mortar on ice. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay.
2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Working Reagent Ⅱ to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay.
3. Plasma, Serum or other Liquid samples: Take 0.5 mL liquid, add 0.5 mL Working Reagent Ⅱ and mix well (equivalent to double dilution). Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 460 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Working Reagent place at 37℃ incubation for 10 min.
3. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Test Well (μL)	Control Well (μL)
Sample	30	30
Reagent Ⅰ	0	270
Working Reagent	270	0
Mix well, Incubate for 30 min at 37℃		
Reagent Ⅴ	3	3

4. Mix well, Incubate for 10 min at 60℃. Centrifuge at 12,000 g for 5 min at room temperature quickly. Take 200 μL into a 96-well plate or microglass cuvette. Detect the absorbance at 460 nm. The Test Well is marked as A_{Test}, the Control Well is marked as

$A_{Control}$. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately or extend the incubation time at 37℃, and divide the calculated result by the corresponding time. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with Working Reagent II, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculation of the MPO activity

A. 96-well plates calculation formula as below

(1) Calculated by protein concentration

Active unit definition: 1 μmol o-Dianisidine producted per min in 1 mg tissue protein reaction system at 37℃ is defined as a unit of enzyme activity.

MPO (nmol/mg prot)=ΔA÷(ε×d)×$V_{反总}$÷(Cpr×$V_{样}$)÷T**=5,386.67×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: 1 μmol o-Dianisidine producted per min in 1 g tissue reaction system at 37℃ is defined as a unit of enzyme activity.

MPO (nmol/g fresh weight)=ΔA÷(ε×d)×$V_{反总}$÷(W÷$V_{样总}$×$V_{样}$)÷T**=5,386.67×ΔA÷W**

(3) Calculated by cells

Active unit definition: 1 μmol o-Dianisidine producted per min in 10^4 cell reaction system at 37℃ is defined as a unit of enzyme activity.

MPO (nmol/10^4 cell)=ΔA÷(ε×d)×$V_{反总}$÷(n÷$V_{样总}$×$V_{样}$)÷T**=5,386.67×ΔA÷n**

(4) Calculated by volume of liquid samples

Active unit definition: 1 μmol o-Dianisidine producted per min in 1 mL liquid reaction system at 37℃ is defined as a unit of enzyme activity.

MPO (nmol/mL)=ΔA÷(ε×d)×$V_{反总}$÷$V_{样}$×F÷T**=5,386.67×ΔA×F**

ε: o-Dianisidine molar extinction coefficien, 7.5×10^{-3} mL/μmol/cm; d: 96-well plate diameter, 0.5 cm; V_{Total}: total reaction volume, 0.303 mL; V_{Sample}: added the sample volume, 0.03 mL; $V_{Total\ sample}$: added the Working Reagent II volume, 1 mL; Cpr: sample protein concentration, mg/mL; T: reaction time, 30 min=0.5 h; W: Sample weight, g; n: Number of cells, calculated in units of ten thousand; F: dilution ratio of liquid sample pretreatment, 2.

B. Microglass cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

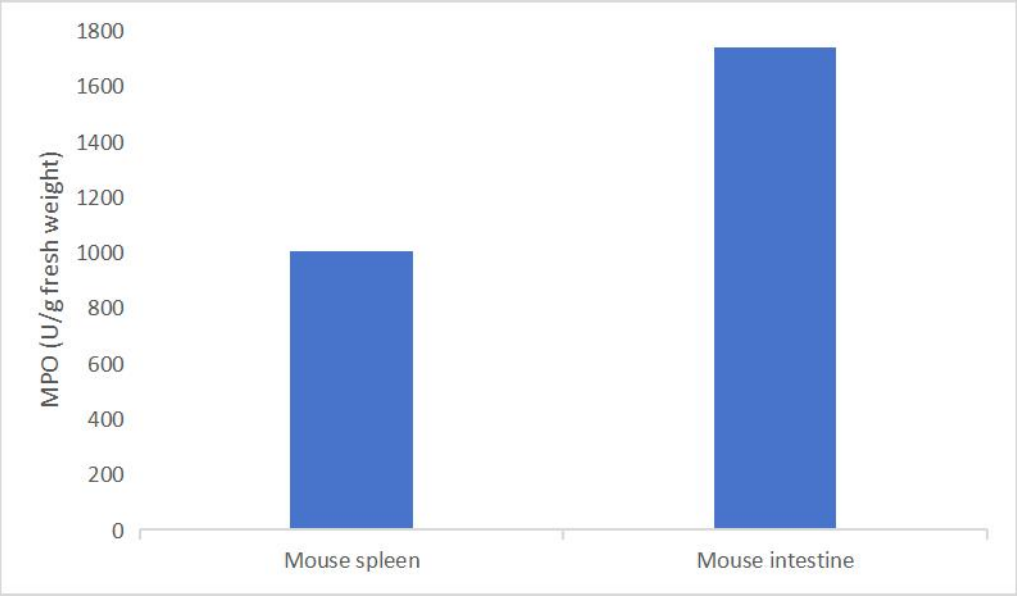

Figure 1. Determination of MPO activity in mouse spleen and intestine by this kit.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.