Myeloperoxidase (MPO) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC5715

Size: 100T/48S

Components:

Reagent I: Liquid 80mL×1. Store at 2-8℃.

Reagent II: Powder×2. Store at 2-8℃. Add 30mL Reagent I before use and dissolve completely at 37℃. It could be stored at 2-8℃ for twelve weeks.

Reagent III: Liquid 3mL×1. Store at 2-8℃. At low temperatures, the reagent may precipitate and solidify. It need to be heated to a clear and transparent state at 37 ℃ before use.

Reagent IV: Liquid 1mL×1. Store at 2-8℃.

Reagent V: Liquid 0.1mL×1. Store at 2-8℃.

Reagent VI: Liquid 1mL×1. Store at 2-8℃.

working solution: Reagent I, Reagent IV and Reagent V are mixed by the ratio of 4.8**mL**：200μL：5μL(5mL, 16S) to make working solution according to sample number before use.

Product Description:

Myeloperoxidase (MPO) is a white blood cell enzyme secreted by activated neutrophils and monocytes/macrophages, mainly present in the phenylalanine blue granules of neutrophils and monocytes/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 biomarker of systemic inflammation and oxidative stress.

MPO catalyzes the decomposition of H_2O_2, while oxidizing ortho anisidine to form a colored substance. There is a characteristic absorption peak at 460nm, and the color depth is linearly related to the activity of MPO.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate, centrifuge, balance, water bath/constant temperature incubator, micro glass cuvette/96 well plate, transferpettor, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to in the literature)

1. **Tissue:** According to the proportion of tissue weight (g): Reagent II volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Reagent II and fully homogenized on ice bath.

Centrifuge at 12000g for 10 minutes at 4℃ to remove insoluble materials and take the

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com

supernatant on ice before testing.

2. **Cells:** According to the proportion of cells number (10^6): Reagent II volume (mL) of 5-10:1 to extract. It is suggested that add 1 mL of Reagent II to 5 million of cells. Use ultrasonication to split cells (place on ice, ultrasonic power 200W, working time 3 seconds, interval 7 seconds, repeat for 3 minutes). Centrifuge at 12000g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.
3. **Liquid samples:** According to the proportion of liquid volume (mL): Reagent II volume (mL) of 1:1 to mix(equivalent to diluting twice). Centrifuge at 12000g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing. (If the measured value is small, the proportion of liquid volume (mL) and Reagent II volume (mL) can be adjusted, such as 2:1 or 3:1.)

II. Determination

1. Preheat Spectrophotometer/Microplate for 30 min, adjust the wavelength to 460 nm and set counter to zero with distilled water in Spectrophotometer.
2. Add reagents in 1.5ml EP tube as the following:

Reagent (μL)	Test tube	Control tube
Sample	20	20
Reagent III	20	20
Working solution	300	-
Distilled water	-	300
Mix and react at 37 ℃ for 30 minutes.		
Reagent VI	5	5

Mix and react for 10min at 60℃. Taking 300μL reaction solution in micro glass cuvette or 96 well plate to measure the absorbance values of each tube at 460nm and record it as A_T or A_C. $\Delta A=A_T-A_C$. Each test tube needs to be equipped with a control tube.

Note: When the temperature is low, the reaction solution may become turbid or solidify. It needs to be heated at 37 ℃ until the reaction solution is clear and transparent before measurement.

III. MPO activity calculation:

1. Protein concentration:

   Definition of unit: Each mg of tissue protein per hour catalyzes the production of 1μmol oxidized ortho anisidine at 37 ℃ is defined as an enzyme activity unit.

   MPO activity (U/mg prot) $=\Delta A\div(\varepsilon\times d)\times V_{RV}\div(Cpr\times V_S)\div T=3.053\times\Delta A\div Cpr$
2. Sample weight:

   Definition of unit: Each g of tissue per hour catalyzes the production of 1μmol oxidized ortho anisidine at 37 ℃ is defined as an enzyme activity unit.

   MPO activity (U/g weight) $=\Delta A\div(\varepsilon\times d)\times V_{RV}\div(W\div V_T\times V_S)\div T=3.053\times\Delta A\div W$
3. Cells:

   Definition of unit: Each 10^6 Cells per hour catalyzes the production of 1μmol oxidized ortho

anisidine

at 37 ℃ is defined as an enzyme activity unit.

MPO activity (U/10^6 cell) =ΔA÷(ε×d)×V_{RV}÷(N÷V_T×V_S) ÷T=3.053×ΔA÷N

4. Liqiud:

Definition of unit: Each mL of liquid per hour catalyzes the production of 1μmol oxidized ortho anisidine at 37 ℃ is defined as an enzyme activity unit.

MPO activity (U/mL volume) =ΔA÷(ε×d)×V_{RV}÷V_S×F÷T=6.106×ΔA

ε: Extinction coefficient of oxidized ortho anisidine, 11.3mL/μmol/cm;

d: Optical path, 1cm;

V_{RV}: Total reaction volume, 0.345mL;

V_S: Add sample volume, 0.02mL;

V_T: The volume of reagent II added during tissue/cell sample processing, 1mL;

F: Dilution ratio of liquid pre-treatment, 2;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

N: Cell number, calculated as 10^6;

T: Enzymatic reaction time, 30min=0.5h.

Note:

1. It is recommended to check whether reagent II and reagent III are clear and transparent before each experiment. If not, they need to be heated at 37 ℃ until the solution is clear and transparent before use.
2. If ΔA is less than 0.01 or the absorbance value of the test tube is close to the control tube, it is recommended to increase sample supernatant volume or extend the enzymatic reaction time at 37 ℃ before determination. If ΔA is more than 0.5 or the absorbance value of the test tube is more than 1.5, it is recommended to dilute the sample supernatant with Reagent II before determination. And modify the calculation formula.

Experimental example:

1. Take 0.1029g mouse lung samples, add 1 mL of Reagent II, grind the homogenate with ice bath. Then operate according to the determination steps, calculate ΔA = A_T-A_C = 0.401-0.088 = 0.313. The result is calculated according to the sample weight:
   MPO activity (U/g weight) = 3.053×ΔA÷W = 9.287 U/g weight.
2. Take 4×10^6 KB cells, add 1 mL of Reagent II, grind the homogenate with ice bath. Then operate according to the determination steps, calculate ΔA = A_T-A_C = 0.094-0.046 = 0.048. The result is calculated according to the number of cells:
   MPO activity (U/10^6 cell) = 3.053×ΔA÷N = 0.037 U/10^6 cell.

3. Take 0.5mL of horse serum sample, add 1 mL of Reagent II, grind the homogenate with ice bath.

Then operate according to the determination steps, calculate $\Delta A=A_T-A_C=0.142-0.049=0.093$. The result
is calculated according to the sample volume:
MPO activity (U/mL volume) = $6.106\times\Delta A$ = 0.568 U/mL volume.

References:

[1] Bradley PP, Priebat DA, Christensen RD. et al. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker [J]. The Journal of Investigative Dermatology, 1982, 78(3): 206-209.

[2] Krawisz J E, Sharon P, Stenson W F. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity [J]. Gastroenterology, 1984, 87(6): 1344-1350.

[3] Tatjana Momić, Zoran Vujčić, Vesna Vasić. Kinetics of inhibition of peroxidase activity of myeloperoxidase by quercetin [J]. International Journal of Chemical Kinetics, 2008, 40(7): 384-394.

Related Products:

BC0090/BC0095	Peroxidase (POD) Activity Assay Kit
BC0170/BC0175	Superoxide Dismutase (SOD) Activity Assay Kit
BC0200/BC0205	Catalase (CAT) Activity Assay Kit
BC0220/BC0225	Ascorbate Peroxidase (APX) Activity Assay Kit

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com