Myeloperoxidase (MPO) Activity Assay Kit

Catalog Number EEA016 (96 tests)

Rev 3.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

This kit can be used to detect myeloperoxidase (MPO) activity in serum, plasma, milk, and animal tissue. Myeloperoxidase is a heme-containing cationic glycoprotein that belongs to the heme peroxidase family in mammals. MPO is a dimer formed by polymerization of two subunits. Each subunit contains a heavy chain and a light chain. MPO is abundant in the azuropathic granules of polymorphonuclear leukocytes (PMNLs) and a small number in monocytes and macrophages. Studies have shown that MPO plays an important role in the generation of oxidants and host defense in neutrophils, and is closely related to the pathogenesis of many diseases, including cardiovascular disease, lung injury, and cancer.

Myeloperoxidase reduces hydrogen peroxide to a complex. The complex reacts 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 460 nm.

Contents and storage

Kit and components are shipped at 2-8℃. An unopened kit can be stored at 2-8℃ for 12 months.

Components	Quantity (96 tests)
Buffer Solution	20 mL
Powder A	Powder × 2 vials
Powder B	Powder × 2 vials
Saline Solution	6 mL
Clarificant	1.2 mL × 2 vials
Powder C	Powder × 2 vials
Substrate	0.1 mL
Acid Reagent	1 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 460 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37 ℃/ 60 ℃ .

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4 ℃, then take the supernatant for detection.

Milk samples: Collect fresh milk sample, centrifuge at 10000 g for 10 min at 4 ℃. Discard the upper white liquid and preserve the middle layer liquid on ice for detection.

Tissue samples:

- Take 0.02-1 g fresh tissue to wash with PBS(0.01 M, pH 7.4) at 2-8 ℃ to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of powder A application solution (mL): the weight of the tissue (g) =19:1. Mechanical homogenate the sample in ice water bath.
- Don't centrifuge, preserve the sample on ice for detection.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (19.42-893.31 U/L).

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
5% Rat kidney tissue homogenate	1
5% Rat spleen tissue homogenate	1

Note: The diluent is powder A application solution.

Preparation of buffer solution application solution

Mix 1 part buffer solution with 9 parts distilled water. The prepared solution can be stored at 2-8 ℃ for 1 month.

Preparation of powder A application solution

Add vial of powder A with 60 mL buffer solution application solution, and heat at 37 ℃ to dissolve, store at 2-8 ℃ for 2 weeks.

Preparation of powder B application solution

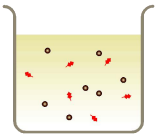
Dissolve vial of powder B with 3 mL Saline Solution. The prepared solution can be store at 2-8 ℃ for 2 weeks.

Preparation of chromogenic agent

Dissolve vial of powder C with 12.5 mL buffer application solution, then add 12.5 μL of Substrate solution. Mix fully and store at 2-8 ℃ with shading light.

Note: If clarificant is frozen, shake in 37℃ water bath to redissolve fully before use.

Assay Protocol



1. Sample pretreatment

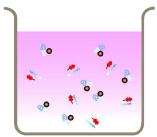
a. Tissue sample: Take 90 μL of tissue homogenate and add 10 μL of powder B application solution, mix fully and incubate at 37 ℃ for 15 min.

b. 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.

2. Add sample and control

a. Sample tube: add 20 μL of sample, 20 μL of clarificant, and 350 μL of chromogenic agent into 1.5 mL microcentrifuge tubes.

b. Control well: add 350 μL of distilled water, 20 μL of sample, and 20 μL of clarificant into 1.5 mL microcentrifuge tubes.



3. Add substrate

a. Oscillate fully with a vortex mixer and incubate for 30 min at 37 ℃.

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

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

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

Calculation

Serum (plasma) and other liquid sample:

Unit definition: the amount of MPO in 1 L of sample that catalyze decomposition of 1 H_2O_2 at 37 ℃ for 30 min is defined as 1 unit.

$$\text{MPO activity (U/L)} = \frac{\Delta A}{11.3^* \times b} \times V_{Total} \div \left(\frac{V_{Sample}}{V_1} \times V_2\right) \times 1000 \times f = \frac{0.175 \times 1000 \times \Delta A}{V_{Sample}} \times f$$

Tissue sample:

Unit 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.

$$\text{MPO activity (U/g wet weight)} = \frac{\Delta A}{11.3^* \times b} \times V_{Total} \div \left(\frac{m}{V_3} \times V_2 \times 0.9\right) = \frac{1.942 \times V_3 \times \Delta A}{m} \times f$$

[Note]

ΔA: OD_{sample}-$OD_{control}$;

11.3*: Constant;

b: Optical path, 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: 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: Dilution factor of sample before tested

To easy calculate the test results, refer to the calculation file available on the webpage.

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:

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

Performance characteristics

- Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	36.50	364.50	579.60
%CV	5.8	5.4	5.0

CV = Coefficient of Variation

- 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	364.50	579.60
%CV	7.2	7.5	7.2

CV = Coefficient of Variation

▪ Expected values

This assay was tested with human serum, and plasma samples at dilutions from 1:10 to 1:60 in Assay Buffer

Sample Type	Range (U/L)	Average (U/L)
Rat serum	400-500	442.5
Human plasma	141-170	153.6

▪ 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 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
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

- Recommended Plate Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'
H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'	S48	S48'
[Note]: S1-S48, sample wells; S1'-S48', control wells.												

- 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.

Limited product warranty

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