TECHNICAL MANUAL

Myeloperoxidase (MPO) Activity Assay Kit

- **SKU CODE:** MAES0076
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Sample pretreatment
- Add samples and reagents
- Measure the OD value

2. Intended use

This kit can be used to detect myeloperoxidase (MPO) activity in serum, plasma, milk, and animal tissue samples.

3. Detection principle

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.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL x 1 vial	20 mL x 1 vial	2-8 °C, 12 months
Reagent 2	Powder A	Powder x 1 vial	Powder x 2 vials	2-8 °C, 12 months
Reagent 3	Powder B	Powder x 1 vial	Powder x 2 vials	2-8 °C, 12 months
Reagent 4	Saline Solution	3 mL x 1 vial	6 mL x 1 vial	2-8 °C, 12 months
Reagent 5	Clarificant	1.2 mL x 1 vial	1.2 mL x 2 vials	2-8 °C, 12 months
Reagent 6	Powder C	Powder x 1 vial	Powder x 2 vials	2-8 °C, 12 months, shading light
Reagent 7	Substrate	0.1 mL x 1 vial	0.1 mL x 1 vial	2-8 °C, 12 months
Reagent 8	Acid Reagent	1 mL x 1 vial	1 mL x 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (460 nm), Tubes, Micropipette, Vortex mixer, 37 °C/60 °C Water bath

Reagents:

Double distilled water

6. 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 °C 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 3 mL 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 12.5 mL of buffer application solution, mix well to dissolve. Add 12.5 µL of substrate, mix well to dissolve. Store at 2-8 °C protected from light.
6. If clarificant freezes in cold condition, shake in 37 °C water bath to dissolve fully (transparent) before use.

7. Sample preparation

Sample preparation

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C 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 powder A application solution with a dounce homogenizer at 4 °C.

4. Collect sample and keep it on ice for detection.

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
Human milk	1
Cell culture supernatant	1
Rat serum	1
Rat plasma	1
10% Rat kidney tissue homogenate	1
10% Rat spleen tissue homogenate	1

Note: The diluent is powder A application solution. For the dilution of other sample types, please perform pretest to confirm the dilution factor.

8. The key points of the assay

1. The supernatant must be clarified after centrifugation during the operation step.
2. If 10% tissue homogenate is used for the experiment and the OD value is very low, 20% tissue homogenate can be used. The reagent 2 application solution is added according to the proportion of tissue weight (g) : volume (mL) = 1:9, and the homogenate time is increased to grind the tissue as thoroughly as possible.
3. Chelating agents such as EDTA should not be added to the sample.

9. Operating steps

Sample pretreatment:

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

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 °C for 15 min.

The measurement of samples:

1. Control tube: Add 350 μL of double distilled water, 20 μL of sample, 20 μL of clarificant into 1.5 mL EP tubes.

Sample tube: Add 20 μL of sample, 20 μL of clarificant, 350 μL of chromogenic agent into 1.5 mL EP tubes.

2. Oscillate fully with a vortex mixer and incubate for 30 min at 37 °C.

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

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

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

10. Calculation

The sample:

1. Serum (plasma) sample:

Definition: The amount of MPO in 1 L of sample that catalyzes decomposition of 1 μmol H_{2O}_2 at 37 °C for 30 min is defined as 1 unit.

MPO activity (U/L) = deltaA / (11.3* x b x V_{Total}) ÷ (V_{Sample} / V_1 x V_2) x 1000 x f

= 0.175 x 1000 x deltaA / V_{Sample} x f

2. Tissue sample:

Definition: The amount of MPO in 1 g wet weight of tissue that catalyzes decomposition of 1 μmol H_{2O}_2 at 37 °C for 30 min is defined as 1 unit.

MPO activity (U/g wet weight) = deltaA / (11.3* x b x V_{Total}) ÷ (m / V_3 x V_2 x 0.9) x f

= 1.942 x V_3 x deltaA / m x f

[Note]

deltaA: 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, 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: The 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: The dilution factor of sample before tested.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	36.50	264.50	579.60
%CV	5.8	5.4	5.0

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	36.50	264.50	579.60
%CV	7.2	7.5	7.2

Recovery

Three samples of high, middle, and low concentration were tested in parallel 6 times each to obtain an average recovery rate of 104%.

Recovery

Sample	Sample 1	Sample 2	Sample 3
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

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.

12. Appendix II Example Analysis

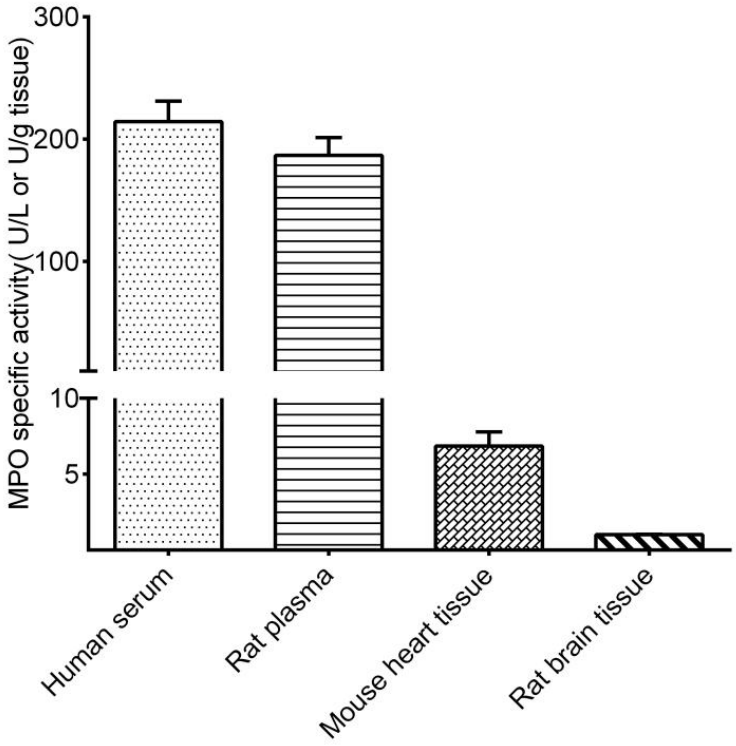
Example analysis:

For human serum, take 45 µL of human serum, and perform 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:

MPO activity (U/L) = (0.116-0.061) / 0.045 x 0.175 x 1000 = 213.89 U/L

Detect human serum (45 µL), rat plasma (45 µL), 5% mouse heart tissue homogenate (90 µL), 5% rat brain tissue homogenate (90 µL) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.