Myeloperoxidase (MPO) Peroxidation Activity Assay Kit

(Catalog #BN00962; 100 assays; Store at -20°C)

I. Introduction:

Myeloperoxidase (MPO: EC 1.11.2.2) is a peroxidase abundantly expressed in neutrophil granulocytes. It catalyzes the hydrogen peroxide (H_2O_2) dependent oxidation of chloride anions (Cl^-) to generate hypochlorite (HClO). During respiratory burst, neutrophils utilize MPO activity to exhibit cytotoxicity against tumor cells and pathogenic microorganisms. MPO also oxidizes various substances such as phenols and anilines, which can lead to inflammation. Increased MPO levels are associated with an increased risk of cardiovascular disease and myocardial infarction. Thus measurement of MPO peroxidation activity is crucial for disease prediction and therapeutics. Assay Genie's Myeloperoxidase (MPO) Peroxidation Activity Assay Kit, oxidizes a substrate to generate fluorescence (Ex/Em = 535/587 nm), which is directly proportional to total peroxidase activity in the sample. A specific MPO inhibitor is provided, which suppresses peroxidase activity due to MPO. This permits differentiation of MPO-mediated peroxidation from the activity of other peroxidases which may be present in the samples. Our kit provides a sensitive, quick and easy method for detecting MPO activity. The assay is high-throughput adaptable and can detect less than 2 µU of MPO activity.

$$H_2O_2 + \text{Peroxidation Substrate} \xrightarrow{\text{Peroxidase}} \text{Fluorescence 1 (Ex/Em= 535/587 nm)}$$

$$H_2O_2 + \text{Peroxidation Substrate + MPO Inhibitor} \xrightarrow{\text{Peroxidase}} \text{Fluorescence 2 (Ex/Em= 535/587 nm)}$$

Fluorescence due to MPO activity = Fluorescence 1 – Fluorescence 2

II. Application:

- Measurement of MPO activity in various tissues/cells
- Screening MPO inhibitors for drug development

III. Sample Type:

- Animal tissues: Liver, Heart, Spleen, etc.
- Cells: Adherent or suspension

IV. Kit Contents:

Components	BN00962	Cap Code	Part Number
MPO Assay Buffer	50 ml	NM	BN00962-1
MPO Peroxidation Substrate (in DMSO)	200 µl	Red	BN00962-2
Hydrogen Peroxide (0.88 M)	50 µl	Blue	BN00962-3
Resorufin Standard (5 mM in DMSO)	50 µl	Yellow	BN00962-4
MPO Positive Control	Lyophilized	Purple	BN00962-5
MPO Inhibitor (4-Aminobenzoic Hydrazide, in DMSO)	100 µl	Orange	BN00962-6

V. User Supplied Reagents and Equipment:

- 96-well flat-bottom tissue culture plate
- 96-well flat-bottom white plate for fluorometric measurement
- Multi-well spectrophotometer (fluorescence plate reader)

VI. Storage, Handling and Reagent Preparation:

Store kit at -20°C, protected from light. Warm the MPO Assay Buffer to room temperature (RT) before use. Briefly centrifuge small vials prior to opening. Store MPO Assay Buffer at 4°C.

- **MPO Peroxidation Substrate, Resorufin Standard and MPO Inhibitor:** Prior to use, bring to RT.
- **Hydrogen Peroxide:** Freshly prepare 5 mM working Hydrogen Peroxide solution by adding 4 µl Hydrogen Peroxide (0.88 M) with 700 µl MPO Assay Buffer. Always prepare fresh.
- **MPO Positive Control:** Reconstitute with 200 µl MPO Assay Buffer. Aliquot and store at -70°C. Keep on ice while in use. Use within two months. Avoid freeze and thaw.

VII. MPO Peroxidation Activity Assay Protocol:

1. **Sample Preparation:** To prepare cell lysate, collect 6 x10^6 cells by centrifugation at 1,000 x g for 5 min. at 4°C. Add 500 µl MPO Assay Buffer to the cell pellet, mix well and incubate on ice for 10 min. Centrifuge at 10,000 x g for 10 min. at 4°C. Collect the supernatant. For Tissues, use perfused tissue (after removing intravascular blood) samples. Wash tissues (~20 mg) with PBS, add 500 µl of MPO Assay Buffer and rapidly homogenize on ice. Centrifuge at 10,000 x g for 10 min. at 4°C. Collect the supernatant. For Positive Control, dilute 1:10 MPO Positive Control by taking 10 µl of MPO Positive Control to 90 µl Assay Buffer, and mix well. For MPO Inhibitor, dilute inhibitor 10 times by adding 50 µl of MPO inhibitor to 450 µl MPO Assay Buffer, and mix well.

   Notes:
   a. We recommend using fresh cell or tissue lysate for the best results. If needed, samples can be saved at -70°C for future experiments.
   b. We recommend adding Protease Inhibitor Cocktail to the MPO Assay Buffer in 1:1000 ratio while preparing the cell/tissue samples to preserve MPO activity.

2. **Resorufin Standard Curve:** Dilute Resorufin Standard (5 mM) by adding 2 µl of Resorufin Standard to 998 µl MPO Assay Buffer (10 pmol/µL), then take 50 µl of diluted Resorufin (10 pmol/µL) into 450 µl MPO Assay Buffer (1 pmol/µL). Add 0, 4, 8, 12, 16, and 20 µl of 1 pmol/µL Resorufin Standard into a series of wells in a 96-well plate to generate 0, 4, 8, 12, 16, and 20 pmol/well of Resorufin Standard. Adjust volume to 60 µl/well with MPO Assay Buffer. If desired, a linear Standard curve can be generated up to 100 pmoles of Resorufin depending on the sample MPO activity.

3. **Assay Procedure:** Add 2-50 µl sample or 2-20 µl of diluted MPO Positive Control into each well; adjust final volume to 50 µl with MPO Assay Buffer. For each sample prepare 2 parallel wells. Add 10 µl of diluted MPO Inhibitor for one set of the samples and add 10 µl of MPO Assay Buffer to the other set of samples.
   Note:
   Samples which do not contain MPO Inhibitor will show total Peroxidase Activity. Samples containing MPO inhibitor suppress MPO and will exhibit non-MPO activity from other peroxidases.

4. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 40 µl Mix containing:

	Reaction Mix
MPO Assay Buffer	37 µl
MPO Peroxidation Substrate	1 µl
Hydrogen Peroxide	2 µl

   Add 40 µl of the Reaction Mix to each well containing Standard, Positive Control and Test Samples. Mix well.

5. **Measurement:** Measure the fluorescence at Ex/Em = 535/587 nm kinetically for 5-20 min at 37°C.
   Note: Incubation time depends on the myeloperoxidase activity in the samples. We recommend measuring the RFU in the sample (RFU_S) and sample with inhibitor (RFU_I) in a kinetic mode, and choose two time points (T1 & T2) in the linear range to calculate the rate of the reaction. The Resorufin standard curve can be read in Endpoint mode (i.e., at the end of the sample incubation time).

6. **Calculation:** Subtract the 0 standard reading from all standard readings. Plot the Resorufin Standard Curve. Calculate Myeloperoxidase activity of the test sample: $\Delta RFU = (RFU_{S(T2)} – RFU_{S(T1)}) – (RFU_{I(T2)} – RFU_{I(T1)})$. Apply the ΔRFU to the Resorufin Standard Curve to get B pmol of Resorufin generated by Myeloperoxidase during the reaction time ($\Delta T = T2 - T1$).

Sample Myeloperoxidase Activity = B/(ΔT X V) x Dilution Factor = pmol/min/ml = µU/ml

Where: **B** is the Resorufin amount from Standard Curve (pmol)
ΔT is the reaction time (min.)
V is the sample volume added into the reaction well (ml)

MPO activity can also be expressed in µU/mg of total protein in the sample.
Unit Definition: A unit of Myeloperoxidase is the amount of enzyme that generates 1.0 µmol of resorufin per min. at pH 7.0 at 37°C.

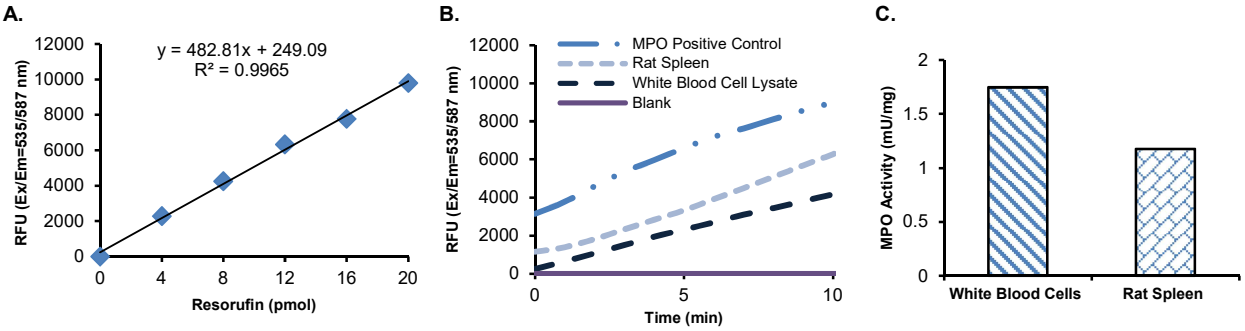

Figure 1: (A). Resorufin Standard Curve (B). Myeloperoxidase Activity (ΔRFU +/- inhibitor) for freshly prepared White Blood Cell Lysate (0.5 µg), Perfused Rat Spleen Lysate (0.9 µg), Positive Control, and reagent blank (C). Calculated Myeloperoxidase Activity in White Blood Cell Lysate and Rat Spleen Lysate. Assays were performed following kit protocol.

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