Technical Manual

FluoroDazzle Myeloperoxidase (MPO) Activity Assay Kit

Catalogue Code: BA0134

Pack Size: 100 assays

Research Use Only

DESCRIPTION

MYELOPEROXIDASE (MPO; EC 1.11.2.2) is a peroxidase enzyme and can be found in neutrophil, monocytes, and some soft tissue macrophages. MPO has an ability to use chloride as a cosubstrate with hydrogen peroxide to generate hypochlorous acid, a powerful antimicrobial agent produced by neutrophils. However, an excessive production of hypochlorous acid can lead to oxidative stress and tissues damage. Inflammation may also result when MPO oxidizes various substances such as phenols and anilines. Studies show that increased MPO levels may increase the risk of myocardial infarction and cardiovascular disease.

The Assay Genie FluoroDazzle Myeloperoxidase (MPO) Activity Assay Kit is based on the MPO enzyme reaction with hydrogen peroxide (H_2O_2) which oxidizes the dye reagent to a highly fluorescent product. The fluorescence intensity of this product, measured at $\lambda_{ex/em}$ = 530/585 nm, is proportional to the total peroxidation activity in the sample. The provided MPO inhibitor is used to suppress peroxidase activity due to MPO in order to differentiate other peroxidase activities that may be present in the samples.

KEY FEATURES

Fast and sensitive. Linear detection range (20 µL sample): 0.0025 to 2 U/L for 10 min reaction at 25˚C.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

MPO peroxidation activity determination in biological samples (e.g. cell lysates, tissues, etc.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	15 mL	**Resorufin:**	1.5 mL
Dye Reagent:	120 µL	**MPO Inhibitor:**	1.5 mL
3% Stabilized H_2O_2:	100 µL		

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at any desired temperature (e.g. 25°C or 37°C).

Sample Preparation:

Tissue: prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) with a Dounce homogenizer in ~200 µL cold 20 mM PBS, pH 7.4. Freeze the homogenized tissue at –80°C to lyse the cells. After freezing, thaw and centrifuge samples at 14,000×*g* for 20 min at 4°C. Remove supernatant for assay.

Cell Lysate: collect cells by centrifugation at 2,000×*g* for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 14,000×*g* for 10 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Reagent Preparation:

Bring all reagents to room temperature prior to assay. Briefly centrifuge tubes before use.

Assay Procedure:

1. Prepare 250 µL 30 µM Resorufin Premix by mixing 15 µL provided Resorufin and 235 µL water.

   Transfer 100 µL assay buffer and 100 µL 30 µM Resorufin into two separate wells of a black flat-bottom 96-well plate.

2. For each sample prepare 2 parallel wells. Add 20 µL of samples to each wells. Add 20 µL of MPO inhibitor for one set of the samples and add 20 µL assay buffer to the other set of samples. Incubate samples at room temperature for 10 min.

3. Prepare 0.07% H_2O_2 by mixing 4.7 µL 3% H_2O_2 with 195.3 µL dH_2O. Then to 0.007% H_2O_2 by mixing 60 µL 0.07% H_2O_2 with 540 µL dH_2O. Use the 0.007% H_2O_2 within one hour.

   Prepare enough Working Reagent (WR) for all reaction wells by mixing, for each 96-well assay, 60 µL Assay Buffer, 1 µL 0.007% H_2O_2 and 1 µL Dye Reagent. Add 60 µL WR to all sample and inhibitor wells. Tap plate briefly to mix.

4. Read fluorescence $\lambda_{ex/em}$ = 530/585 nm at 0 min and 10 min at room temperature.

CALCULATION

The MPO activity in a sample is computed as follows:

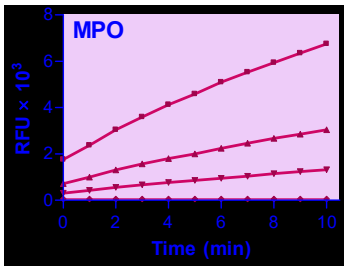
$$\text{MPO Activity} = \frac{\Delta R_{SAMPLE} - \Delta R_{INB}}{R_{RESORUFIN} - R_{H2O}} \times \frac{[\text{Resorufin}]\ (\mu M)}{t\ (\text{min})} \times \frac{\text{Reaction Vol}\ (\mu L)}{\text{Sample Vol}\ (\mu L)} \times n$$

$$= \frac{\Delta R_{SAMPLE} - \Delta R_{INB}}{R_{RESORUFIN} - R_{H2O}} \times 15 \times n \quad (U/L)$$

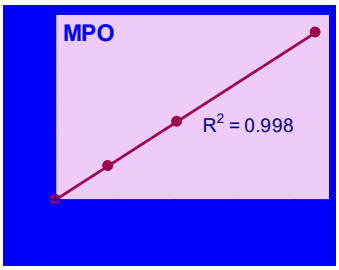
where R_{SAMPLE}, R_{INB}, $R_{RESORUFIN}$ and R_{H2O} are fluorescence readings of the Sample, Sample Inhibitor, Resorufin and Water wells, respectively. $\Delta R_{SAMPLE} = R_{Sample,10min} - R_{Sample,0min}$ and $\Delta R_{INB} = R_{INB,10min} - R_{INB,0min}$. *n* is the sample dilution factor. [Resorufin] = 30 µM, Reaction Vol = 100 µL, Sample Vol = 20 µL, Reaction time (t) = 10 min.

Notes: if ΔR_{SAMPLE} values are higher than that of the $R_{RESORUFIN}$, dilute sample in Assay Buffer and repeat the assay. Multiply the results by the dilution factor, *n*.

Unit definition: one unit of enzyme will catalyze the formation of 1 µmole resorufin per min under the assay conditions.



Raw Kinetic Data



MPO Titration

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, black 96-well plates (e.g. Greiner Bio-One, cat# 655900) and plate reader capable of measuring fluorescence at $\lambda_{ex/em}$= 530/585 nm.

LITERATURE

1. Nicholls, J., et al. (2005) Myeloperoxidase and cardiovascular disease. *Arteriosclerosis, thrombosis, and Vascular Biology* 25.6: 1102-11112.

2. Zhang, R., et al. (2001) Association between myeloperoxidase levels and risk of coronary artery disease. *Jama* 286.17: 2136-2142.

3. Canseven, G., et al. (2008) Effects of various extremely low frequency magnetic fields on the free radical processes, natural antioxidant system and respiratory burst system activities in the heart and liver tissues. *Indian journal of biochemistry & biophysics* 45.5: 326.

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