**Myeloperoxidase Inhibitor Screening Assay Kit**

Cat. No. CTG-BK032S 1 kit

**Product Description**

Myeloperoxidase (MPO) Inhibitor Screening Assay Kit is a rapid, simple, sensitive, and reliable assay suitable for use as a high throughput MPO activity inhibitor screening. In the MPO assay protocol, myeloperoxidase produces HClO from H2O2 and Cl-. Myeloperoxidase (MPO) is a peroxidase enzyme (EC 1.11.1.7) most abundantly present in neutrophil granulocytes. It is a green hemoprotein found in neutrophils and monocytes that catalyzes the reaction of hydrogen peroxide and halide ions to form cytotoxic acids and other intermediates that play a role in the oxygen-dependent killing of tumor cells and microorganisms. Its heme pigment causes the green color in secretions rich in neutrophils, such as some forms of mucus. Furthermore, it can oxidize tyrosine to a tyrosyl radical using hydrogen peroxide as an oxidizing agent. Celltechgen’s MPO Inhibitor Screening Assay provides convenient fluorescence-based methods for screening inhibitors to both the chlorination and peroxidation activities of MPO. The chlorination assay utilizes the non-fluorescent 2-[6-(4-aminophenoxy)-3-oxo-3H-xanthen-9-yl]-benzoic acid (APF), which is selectively cleaved by hypochlorite (-OCl) to yield the highly fluorescent compound fluorescein. Fluorescein fluorescence is analyzed with an excitation wavelength of 480-490 nm and an emission wavelength of 515-520 nm. The peroxidation assay utilizes the peroxidase component of MPO, where a single two electron oxidation of native enzyme (MPO) to compound I (MPO-I) is followed by two successive one electron reductions back to native enzyme by compound II (MPO-II). The reaction between hydrogen peroxide and ADHP (10-acetyl-3,7-dihydroxyphenoxazine) produces the highly fluorescent compound resorufin. Resorufin fluorescence is analyzed with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm. The assay schemes are outlined in Figure below.



**Kit Size**

100 rxns

**Kit Components**

MPO Assay Buffer 50 ml

MPO Chlorination Substrate 100 µl

MPO Peroxidation Substrate (in DMSO) 100 µl

Hydrogen Peroxide (0.88 M) 50 µl

MPO Inhibitor 50 µl

Myeloperoxidase Control 50 µl

**Kit storage/stability**

Store kit at -20°C protected from light. Allow Assay Buffer to warm to room temperature

before use. Briefly centrifuge small vials before opening.

**Usage**

Celltechgen provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please request the Safety Data Sheet (SDS) provided for the product.

**Experimental Protocol**

**1. Prepare solutions**

1. Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.
2. MPO Assay Buffer – Allow buffer to come to room temperature before use.
3. MPO Chlorination Substrate

The vial contains 100 µl of 2.5 mM 2-[6-(4-aminophenoxy)-3-oxo-3H-xanthen-9-yl]-benzoic acid (APF) in DMSO. It is ready to use to prepare the Chlorination Working Solution.

1. MPO Peroxidation Substrate

Each vial contains 100 µl of 5 mM ADHP (10-acetyl-3, 7-dihydroxyphenoxazine) in DMSO. Immediately prior to preparing the Peroxidation Working Solution, dissolve the contents of one vial with 100 µl of MPO DMSO and then add 400 µl of Assay Buffer for a final concentration of 1 mM. This is enough Substrate to assay 100 wells. Prepare additional vials as needed. The reconstituted Substrate is stable for 30 minutes. After 30 minutes, increased background fluorescence will occur.

1. Myeloperoxidase Control

The vial contains 50 µl of a 100 µg/ml solution of human polymorphonuclear leukocyte MPO. Thaw and store the enzyme on ice while preparing the reagents for the assay. Prior to use, dilute 10 µl of MPO with 3.99 ml of Assay Buffer for a final MPO concentration of 300 ng/ml. This is enough enzyme to assay 80 wells. The diluted enzyme is stable for one hour on ice.

1. MPO Inhibitor

The vial contains 300 µl of 50 mM 4-aminobenzhydrazide, a MPO inhibitor. The Inhibitor’s use is optional but may be used to standardize the assay. Prior to use, dilute 10 µl of Inhibitor with 490 µl of Assay Buffer. This is enough Inhibitor to assay 50 wells. The diluted Inhibitor is stable for four hours.

1. MPO Hydrogen Peroxide

The vial contains 100 µl of a 30% solution of hydrogen peroxide. Prior to use, dilute 10 µl with 90 µl of Assay Buffer to yield a 3% solution. Prepare a 5 mM solution by diluting 10 µl of the 3% solution with 1.74 ml of Assay Buffer. The 5 mM solution will be used to prepare the Working Solutions. The diluted solutions are stable for two hours.

**5. Assay Protocol**

**A. Notes**

1. Chlorination and peroxidation activities cannot be measured simultaneously. There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as 100% initial activity and three wells designated as background wells. We suggest that each inhibitor sample be assayed in triplicate.
2. The final volume of the assay is 100 µl in all the wells.
3. We recommend assaying samples in triplicate, but it is the user’s discretion to do so.
4. Both assays are performed at room temperature.
5. Chlorination and peroxidation activities cannot be measured simultaneously.
6. Monitor the Chlorination fluorescence using an excitation wavelength of 480-490 nm and an emission wavelength of 515-520 nm.
7. Monitor the Peroxidation fluorescence using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

**B. Performing the Chlorination Assay**

1. In a suitable tube, prepare the Chlorination Working Solution according to the table below. The solution will turn yellow.

|  |  |  |
| --- | --- | --- |
| **Component** | **50 wells** | **100 wells** |
| Assay Buffer | 1.94 ml | 3.88 µl |
| Chlorination Substrate (2.5 mM) | 40 µl | 80 µl |
| Hydrogen Peroxide (5 mM) | 20 µl | 40 µl |

1. 100% Initial Activity Wells - add 10 µl of Assay Buffer and 50 µl of 250 ng/ml MPO to three wells.
2. Background Wells - add 60 µl of Assay Buffer to three wells.
3. Sample (inhibitor) Wells - add 10 µl of sample (inhibitor) and 50 µl of 250 ng/ml MPO to three wells.
4. Initiate the reactions by quickly adding 40 µl of the Chlorination Working Solution to all of the wells being used.
5. Cover the plate with the plate cover and incubate on a shaker for 10 minutes at room temperature.
6. Remove the plate cover. Read the plate using an excitation wavelength of 480-490 nm and an emission wavelength of 515-520 nm.

Sample (inhibitors) can be dissolved in ethanol, methanol, or DMSO but need to be further diluted into Assay Buffer before being added to the assay in a final volume of 10 µl. Solvents dramatically interfere with the assay. In the event that the appropriate concentration of inhibitor needed for MPO inhibition is completely unknown, we recommend that several concentrations of the compound be assayed.

**C. Performing the Peroxidation Assay**

1. In a suitable tube, prepare the Peroxidation Working Solution according to the table below:

|  |  |  |
| --- | --- | --- |
| **Component** | **50 wells** | **100 wells** |
| Assay Buffer | 1.74 ml | 3.48 ml |
| Peroxidation Substrate (1 mM) | 250 µl | 500 µl |
| Hydrogen Peroxide (5 mM) | 10 µl | 20 µl |

1. 100% Initial Activity Wells - add 10 µl of Assay Buffer and 50 µl of 250 ng/ml MPO to three wells.
2. Background Wells - add 60 µl of Assay Buffer to three wells.
3. Sample (inhibitor) Wells - add 10 µl of sample (inhibitor) and 50 µl of 250 ng/ml MPO to three wells.
4. Initiate the reactions by quickly adding 40 µl of the Peroxidation Working Solution to all of the wells being used.
5. Cover the plate with the plate cover and incubate on a shaker for 5 minutes at room temperature.
6. Remove the plate cover. Read the plate using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

Sample (inhibitors) can be dissolved in ethanol, methanol, or DMSO but need to be further diluted into Assay Buffer before being added to the assay in a final volume of 10 µl. Solvents dramatically interfere with the assay. In the event that the appropriate concentration of inhibitor needed for MPO inhibition is completely unknown, we recommend that several concentrations of the compound be assayed.

**6. Data Analysis**

1. Determine the average fluorescence of the 100% Initial Activity, background, and inhibitor wells.
2. Subtract the fluorescence of the background wells from the fluorescence of the 100% initial activity and inhibitor wells.
3. Determine the percent inhibition for each inhibitor. To do this, subtract each inhibitor sample value from the 100% initial activity sample value. Divide the result by the 100% initial activity value and then multiply by 100 to give the percent inhibition.

% Inhibition = [ Initial Activity – Inhibitor ]/Initial Activity x 100

If multiple concentrations of inhibitor are tested, graph either the Percent Inhibition or Percent Initial Activity as a function of the inhibitor concentration to determine the IC50 value (concentration at which there was 50% inhibition). Examples of MPO chlorination and peroxidation inhibition by the MPO inhibitor, 4-aminobenzhydrazide are shown in Figures 2.

