Bring all reagents to room temperature prior to assay.

**Assay Procedure:**

1. Prepare 250 µL 30 µM Resorufin Premix by mixing 15 µL provided Resorufin and 235 µL water.
2. Transfer 100 µL assay buffer and 100 µL 30 µM Resorufin into two separate wells of a black flat-bottom 96-well plate.
3. For each sample prepare 2 parallel wells. Add 20 µL of samples to each well. Add 20 µL of 1× MPO inhibitor to one of each sample’s wells and add 20 µL assay buffer to the other well. Incubate samples at room temperature for 10 min.
4. Prepare 0.07% H₂O₂ by mixing 4.7 µL 3% H₂O₂ with 195.3 µL dH₂O. Then to 0.007% H₂O₂ by mixing 60 µL 0.07% H₂O₂ with 540 µL dH₂O. Use the 0.007% H₂O₂ within one hour.
5. Prepare enough Working Reagent (WR) for all reaction wells by mixing, for each 96-well assay, 60 µL Assay Buffer, 1 µL 0.007% H₂O₂ and 1 µL Dye Reagent. Add 60 µL WR to all sample and inhibitor wells. Tap plate briefly to mix.
6. Read fluorescence \( \lambda_{530} = 530/585 \) nm at 0 min and 10 min at room temperature.

**CALCULATION**

The MPO activity in a sample is computed as follows:

\[
MPO\ Activity = \frac{\Delta R_{SAMPLE} - \Delta R_{H2O}}{R_{RESORUFIN} - R_{H2O}} \times \frac{\text{[Resorufin] (µM)}}{\text{Reaction Vol (µL)}} \times \frac{\text{Sample Vol (µL)}}{n} \times n \\
= \frac{\Delta R_{SAMPLE} - \Delta R_{H2O}}{R_{RESORUFIN} - R_{H2O}} \times 15 \times n \text{ (U/L)}
\]

where \( R_{SAMPLE} \), \( R_{H2O} \), \( R_{RESORUFIN} \) 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 \( \Delta R_{INH} = R_{INH,10min} - R_{INH,0min} \). \( n \) is the sample dilution factor. \([\text{Resorufin}] = 30 \mu M, \text{Reaction Vol} = 100 \mu L, \text{Sample Vol} = 20 \mu L, \text{Reaction time (t)} = 10 \text{ min.}

**NOTES:**

- if \( \Delta R_{SAMPLE} \) 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.

**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 \( \lambda_{530/585} \) nm.

**LITERATURE**