amsbio

For research use only

Myeloperoxidase (MPO) Colorimetric Activity Assay Kit

(Catalog #K744-100; 100 assays; Store kit at -20°C)

I. Introduction:

Myeloperoxidase (MPO) is a peroxidase enzyme (EC 1.11.1.7) most abundantly expressed in neutrophil granulocytes. It is a lysosomal protein stored in the azurophilic granules of the neutrophil. MPO contains a heme pigment which causes its green color in secretions rich in neutrophils, such as pus and some forms of mucus. MPO catalyzes the production of hypochlorous acid (HClO) from hydrogen peroxide (H_2O_2) and chloride anion (Cl^-, or the equivalent from a non-chlorine halide). MPO also oxidizes tyrosine to a tyrosyl radical using hydrogen peroxide as an oxidizing agent. In BioVision's MPO Assay Kit, the HClO produced from H_2O_2 and Cl^- is reacted with taurine to generate the taurine chloramine, which subsequently reacts with the TNB^{2-} probe to eliminate color (λ = 412 nm). The kit provides a rapid, simple, sensitive, and reliable test suitable for high throughput activity assay of MPO. This kit can be used to detect MPO as low as 0.05 mU per well.

II. Kit Contents:

Component	100 Assays	Cap Code	Part Number
MPO Assay Buffer	25 ml	WM	K744-100-1
TNB Probe (lyophilized)	1 vial	Red	K744-100-2
MPO Substrate Stock	50 µl	Blue	K744-100-3
Stop Mix (lyophilized)	1 vial	Green	K744-100-4
TNB Standard (2.5 µmol; lyophilized)	1 vial	Amber	K744-100-5
MPO Positive Control (lyophilized)	1 vial	Purple	K744-100-6

III. Storage and Handling:

Store the kit at -20 °C protected from light. Allow the Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. **Read the entire protocol prior to performing the assay**.

IV. Reagent Preparation:

TNB Probe: Reconstitute with 1.05 ml dH_2O. Store -20°C. Use within two months.
MPO Substrate: Add 5 µl MPO Substrate Stock into 300 µl dH_2O to prepare the MPO substrate working solution. Aliquot and store at -20°C. The working solution is stable for one week.

Stop Mix: Reconstitute with 220 µl dH_2O, briefly mix. Aliquot and store at -20°C. Use within two months.

TNB Standard: Dissolve TNB standard with 0.5 ml Assay Buffer to generate 5 mM TNB Standard. The TNB standard solution is stable for 1 week at 4°C and 1 month at -20°C.

MPO Positive Control: Reconstitute the positive control with 100 µl MPO Assay Buffer. Aliquot and store at -20 °C. Use within two months.

V. MPO Assay Protocol:

1. **Standard Curve Preparation:**

   Dilute the TNB Standard 1:4 with Assay Buffer to generate a 1 mM solution. Add 0, 2, 4, 6, 8, 10 µl of TNB Standard into a series of wells. Adjust volume to 150 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of TNB Standard. Read standard curve after 10 min at OD 412 nm.

2. **Sample Preparations:**

   Tissues or cells can be homogenized in 4 volumes of Assay Buffer, centrifuged (13,000 x g, 10 min) to remove insoluble material. Serum samples can be directly diluted in the Assay Buffer. Prepare test samples of up to 50 µl/well with Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to ensure the readings are within the standard curve range.

3. **Positive Control (optional):**

   Add 5 - 10 µl of the reconstituted MPO Positive Control into the Positive Control well(s) and adjust the final volume to 50 µl/well with MPO Assay Buffer.

4. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 90 µl Reaction Mix:

MPO Measurement	Sample Background Control
80 µl MPO Assay Buffer	80 µl MPO Assay Buffer
10 µl MPO Substrate	10 µl dH_2O

5. Add 90 µl of the Reaction Mix to each well containing the Positive Controls and Samples. Add 90 µl of the Sample Background Control Mix to the sample background controls. Mix well. Incubate at room temperature for 30 min to 2 hr (record this time as **T**), then add 2 µl Stop Mix and mix well. Incubate another 10 min to stop the reaction then add 10 µl TNB Probe. Mix well. It is suggested to run samples for 30 min, 1 hr and 2 hr. Followed by the Stop Mix and TNB Probe additions at each time point-to ensure values will fall within the linear range of the Standard Curve.

   (**DO NOT ADD REACTION MIX OR STOP MIX TO STANDARDS**)

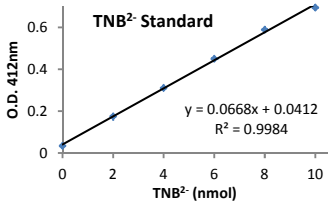
6. **Sample Measurement:** After 5 min, read the Positive Control(s) and samples OD at 412 nm. The OD of color upon decrease of TNB is $\Delta A_{412nm} = A_{background} - A_{sample}$. It is recommended to use the ΔA values which are in the linear range of the Standard Curve.

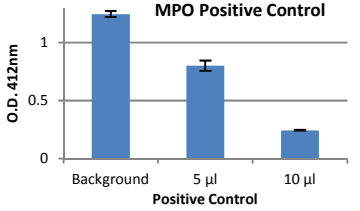
7. **Calculation:** Subtract the 0 Standard reading from all standard readings. Plot the Standard Curve. Apply the $\Delta A_{412\ nm}$ of samples to the Standard Curve to get B (nmol of TNB consumed in the sample reaction between the given time). MPO activity in samples can then be calculated:

$$\textbf{MPO Activity} = \frac{\mathbf{B}}{\mathbf{T} \times \mathbf{V}} \times \textbf{Sample Dilution Factor} = \textbf{nmol/min/ml} = \textbf{mU/ml}$$

Where: **B** is the TNB amount calculated from the Standard Curve (in nmol).
T is the time of the first incubation (**i.e., pre-Stop Mix, in min**).
V is the pre-adjusted sample volume added into the reaction well (in ml).

Unit Definition: One unit of MPO is defined as the amount of MPO which hydrolyzes the substrate and generates taurine chloramine to consume 1.0 µmol of TNB per minute at 25 °C.





VI. Related Products:

NAD/NADH Quantification Kit
Fatty Acid Assay Kit
Triglyceride Assay Kit
Lipase Assay Kit
Adipogenesis Assay Kit
Lactate assay Kits
Glycogen Assay Kit
Creatine & Creatinine Assay Kits
Amino Acid Assay Kits
NADP/NADPH Quantification Kit
Uric Acid Assay Kit
Free Glycerol Assay Kit
Ethanol Assay Kit
Cholesterol Assay Kits
Pyruvate Assay Kit
Glucose Assay Kits
Sarcosine Assay Kit
Protein Quantitation Kit

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note# The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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