APExBIO
An Apoptosis and Epigenetics Company

Product Information

Myeloperoxidase (MPO) Colorimetric Activity Assay Kit

I. Kit Contents:

Components	K2168-100 100 assays	Cap Color	Part Number
MPO Assay Buffer	25 ml	WM	K2168-C-1
DTNB Probe (100 mM)	50 µl	Red	K2168-C-2
TCEP (50 mM)	50 µl	Clear	K2168-C-3
MPO Substrate	50 µl	Blue	K2168-C-4
Stop Mix	20 µl	Green	K2168-C-5
MPO Positive Control (lyophilized)	1 vial	Purple	K2168-C-6

II. Introduction:

Myeloperoxidase (MPO) is a peroxidase enzyme that expressed most abundantly in neutrophil granulocytes. It is a liposomal protein located in the azurophilic granules of the neutrophil. MPO catalyzes the conversion of hydrogen peroxide (H_2O_2) and chloride anion (Cl-) to hypochlorous acid (HClO). MPO oxidizes tyrosine to a tyrosyl radical utilizing hydrogen peroxide as an oxidizing agent. In Myeloperoxidase (MPO) Colorimetric Activity Assay Kit, HCIO reacted with taurine to produce the taurine chloramine, that subsequently reacts with the TNB2- probe to emit color (λ = 412 nm). This kit offers a reliable and sensitive way for high throughput activity assay of MPO. The detection limit can be as low as 0.05 mU per well.

III. Storage and Handling:

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

IV. Reagent Preparation:

TNB Reagent/Standard: TNB Reagent/Standard: TNB is easily oxidizable so it needs to be prepared from DTNB Probe as needed. Use the same day as prepared, discard any unused TNB reagent/standard. The amount of DTNB Probe for each well (standard, sample and background control) is 0.5µl. The amount per well of TCEP is 0.5 µl and of Assay Buffer is 49 µl for a total of 50 µl per well. (Example: For 10 wells, take 5 µl DTNB Probe, 5 µl TCEP and 490 µl Buffer, mix and set aside.)

MPO Substrate: Aliquot and store at -20℃. Stable for 2 months. Working solution: Add 5 µl MPO Substrate to 300 µl dH_2O. Make fresh and discard unused portion.

Stop Mix: Add 200 µl dH_2O and dissolve. Aliquot and store at -20℃. Use within two months.

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

V. MPO Assay Protocol:

1. Standard Curve Preparation: Add 150, 140, 130, 120, 110 and 100 µl of MPO Assay Buffer into a series of wells. The Standard will be added to the wells (0, 10, 20, 30, 40, 50 µl respectively) at the end of the sample incubation period (see (4) below).
2. Sample Preparation: Homogenize tissue or cells in 4 volumes of PBS having 0.1 % NP40, centrifuge (13,000g, 10 min) to remove insoluble material. Serum samples can be directly diluted in the MPO Assay Buffer. Add 1-50 µl test samples in 96 well plate. For white blood cells, take 2 ml

of blood and lyse RBC using RBC Lysis Buffer. Incubate for 10 min. at room temperature. Centrifuge at 400 x g for 5 min. and remove the supernatant carefully. Wash the pellet with 1 ml 1X PBS. Centrifuge at 400 x g for 5 min, and remove the supernatant carefully. Lyse the pellet using 200 µl MPO Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 x g for 10 min. to remove insoluble material. Collect the supernatant. Add 1 - 10 µl of the WBC lysate into a 96-well plate. Prepare parallel sample well(s) as background control. Adjust the volume of background control and sample wells to 50 µl/well with Assay Buffer. We suggest testing several doses of a sample to ensure the readings are within the standard curve range.

3. Positive Control Preparation: Add 5 - 10 µl of the reconstituted MPO Positive Control to optional Positive Control well(s). Adjust the final volume 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 50 µl Reaction Mix:

	Reaction Mix Sample	Background Control Mix
MPO Assay Buffer	40 µl	40 µl
MPO Substrate	10 µl	10 µl

Add 50 µl of the Reaction Mix to each well containing the Samples & Positive Control wells. Add 50 µl of the Sample Background Control Mix to the sample background controls. Mix well. Note: Do Not Add Reaction Mix To Standards. Incubate at 25℃ for 30 to 120 min. (record this time as T), then add 2 µl Stop Mix to all sample, Standard wells, background control & Positive Control wells and mix. Incubate another 10 min to stop the reaction & add 50 µl TNB Reagent/Standard to each of the sample, sample background control & Positive Control wells. Add 0-10-20-30-40-50 µl TNB Reagent/Standard (0-10-20-30-40-50 nmol respectively) to the Standard wells at this time. We suggest running samples for 30, 60 and 120 min followed by the Stop Mix and TNB Reagent at each time point to ensure values will fall within the linear range of the Standard Curve.

5. Measurement: After 5 - 10 min, read at 412 nm. The Positive Controls and samples will show decreased color proportional to the amount of enzyme present, calculated as $\Delta A412nm = A_{sample\ background} - A_{sample}$. It is recommended to use the ΔA values which are in the linear range of the Standard Curve.

6. Calculation: Subtract 0 Standard reading from all Standard readings. Plot the TNB Standard Curve. Apply the ΔA412 nm of samples to the Standard Curve to get B nmol of TNB consumed in the sample reaction during the given time.

Sample MPO Activity = $B/(T \times V) \times$ Sample Dilution Factor = nmol/min/ml = mU/ml

Where: B = TNB amount calculated from the Standard Curve (in nmol).

T = time of the first incubation (i.e., pre-Stop Mix, in min).

V = 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 generates taurine chloramine to consume 1.0 µmol of TNB per minute at 25℃.

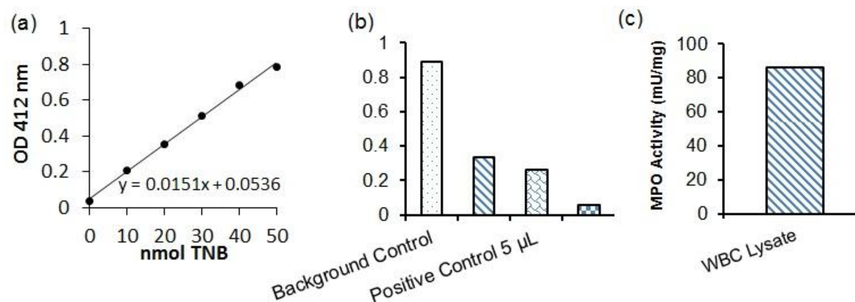

Figure: (a) TNB Standard Curve. (b) Measurement of MPO activity using WBC lysate (3 µg), and MPO Positive Control (5 µl) and (10 µl). (c) MPO specific activity in WBC lysate. Assays were performed following kit protocol.

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General Troubleshooting Guide:

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

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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