APExBIO
An Apoptosis and Epigenetics Company

Product Information

Myeloperoxidase (MPO) Colorimetric Activity Assay Kit

I. Kit Contents:

Components	K2169-100 100 assays	Cap Color	Part Number
MPO Assay Buffer	25 ml	WM	K2169-C-1
MPO Substrate Stock	50 µl	Blue	K2169-C-2
MPO Probe	200 µl	Red	K2169-C-3
Fluorescein Standard (1 mM)	50 µl	Yellow	K2169-C-4
MPO Positive Control	1 vial	Purple	K2169-C-5

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 and alide ions to cytotoxic acids and other intermediates that play a role in the oxygen-dependent killing of tumor cells and microorganisms.. MPO oxidizes tyrosine to a tyrosyl radical utilizing hydrogen peroxide as an oxidizing agent. In Myeloperoxidase (MPO) Fluorometric Activity Assay Kit, MPO catalyzes reaction of H_2O_2 and NaCl to produce NaClO that subsequently reacts with the Aminophenyl fluorescein (APF) to generate detectable strong fluorescence (Ex/Em = 485/525 nm). This kit offers a reliable and sensitive way for high throughput activity assay of MPO. The detection limit can be as low as 0.5 µU per well.

III. Storage and Handling:

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

IV. Reagent Preparation:

MPO Substrate: Mix 4 µl MPO Substrate Stock with 700 µl Assay Buffer to prepare the MPO Substrate Solution.

MPO Positive Control: Reconstitute the MPO Positive Control with 50 µl Assay Buffer. Aliquot and store at -20°C. Use within one month. Avoid freeze thaw cycles.

V. MPO Assay Protocol:

1. Standard Curve Preparation: Mix 5 µl of 1 mM Fluorescein Standard with 995 µl of Assay Buffer to prepare a 5 µM Fluorescein Standard solution. Add 0, 2, 4, 6, 8, 10 µl of 5 µM Fluorescein Standard solution into a series of wells. Adjust volume to 100 µl/well with Assay Buffer to generate 0, 10, 20, 30, 40, 50 pmol/well of Fluorescein Standard. Mix well. Read the Standard Curve at Ex/Em = 485/525 nm after 5 min.
2. Sample Preparations: Tissues or cells can be homogenized in 4 volumes of Assay Buffer and 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. 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

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Buffer. Keep on ice for 10 min. Centrifuge at 10,000 x g for 10 min. to remove insoluble material. Collect the supernatant. Dilute the supernatant 10 times with MPO Assay Buffer and add 1 - 10 µl of the diluted WBC lysate into a 96-well plate. Adjust the volume to 50 µl with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

3. Positive Control (optional): Add 10 µl of the reconstituted MPO Positive Control into Positive Control wells and adjust the volume to 50 µl/well with 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:

MPO Assay Buffer	46 µl
MPO Substrate Solution	2 µl
MPO Probe	2 µl

Add 50 µl of the Reaction Mix to each well containing the Positive Controls and Samples. Mix well. (Do Not Add To Standards)

5. Sample Measurement: Read Ex/Em = 485/525 nm R1 at T1. Read R2 again at T2 after incubating the reaction at room temperature for 30 min (or longer time if the Sample's activity is low); protect from light. The amount of RFU generated in the time interval T2 - T1 is ΔRFU = R2 - R1. It is recommended to read kinetically to choose the R1 and R2 within the linear range. Plot the Fluorescein Standard Curve and apply the ΔRFU to the Standard Curve to get B pmol of fluorescein (amount of fluorescein generated between T1 and T2 in the reaction wells). MPO activity can then be calculated:

MPO Activity = B/ (T× V) × Sample Dilution Factor = pmol/min/ml = µU/ml

Where: B is the fluorescein amount from the Standard Curve (in pmol).

T1 is the time of the first reading (R1) (in min).

T2 is the time of the second reading (R2) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

One unit is defined as the amount of Myeloperoxidase that oxidizes the substrate to yield 1.0 µmol of fluorescein per minute at room temperature.

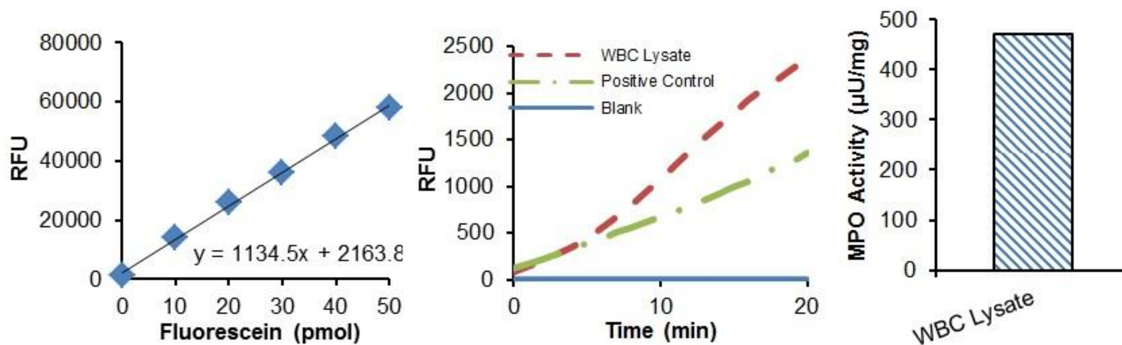

Figure: a) Fluorescein Standard Curve. b) Measurement of MPO activity in WBC lysate (0.1 µg), and MPO Positive Control (3 µl). c) MPO specific activity in WBC lysate. Assays were performed following the kit protocol.

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

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