Version 3 Last updated 1 May 2019

ab236210 Respiratory Burst Assay Kit (Neutrophil/Monocyte)

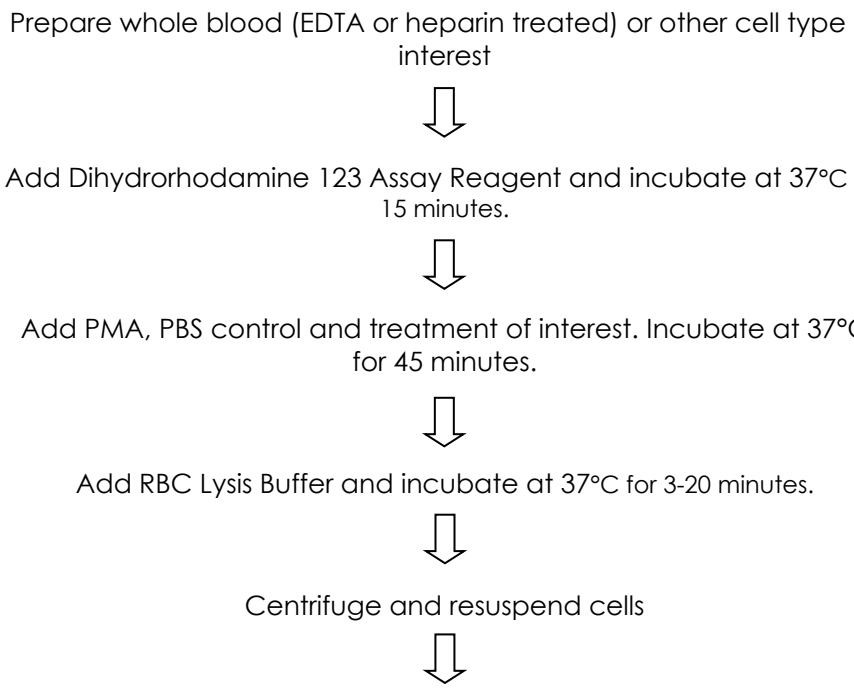
For the measurement of NADPH oxidase-dependent respiratory burst response in whole blood and a range of cell types.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Respiratory Burst Assay Kit (Neutrophil/Monocyte) (ab236210) provides PMA, dihydrorhodamine 123, and additional reagents necessary for inducing and quantifying a respiratory burst response in neutrophils and monocytes by flow cytometry. The assay can be performed on whole blood or on cells in various types of cell culture media. The dihydrorhodamine 123 used in this assay, a cell-permeable, non-fluorescent dye, is converted to the fluorescent compound rhodamine 123 by reactive species produced by activated phagocytes to destroy invading microorganisms. The assay has been validated in human and mouse whole blood, or using other sources of leukocytes. Because the assay reagents are not species-specific, this assay can be used in any species or cell type capable of producing a NADPH oxidase-dependent respiratory burst response.



2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Bovine Serum Albumin Assay Reagent	5 g	4°C	N/A
Calcium Chloride (1 M) Assay Reagent	1 mL	Room temperature	Room temperature
Cell-Based PMA (1 mM)	50 µL	-20°C	Do not store
Dihydrorhodamine 123 Assay Reagent	50 µL	-20°C	Do not store
RBC Lysis Buffer (10X)	10 mL	4°C	4°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- RPMI 1640 cell culture medium
- Source of human or animal blood or leukocytes
- PBS
- Swinging-bucket tabletop centrifuge
- 12 x 75 mm polypropylene test tubes
- Flow cytometer

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 RBC Lysis Buffer (10X)

1. On the day of use, combine 1 ml of RBC Lysis Buffer (10X) with 9 mL of distilled water.
2. Warm to room temperature prior to use.
3. Discard the reagent after 48 hours.
4. The remaining unused RBC Lysis Buffer (10X) can be stored for 1 year at 4°C.

5.2 Cell-Based PMA (1 mM)

1. The vial contains 50 µL of 1 mM PMA in DMSO. Thaw at room temperature.
2. Dilute a portion of the DMSO stock solution 1:1,000 in Assay Buffer to make a 5X working solution. Any unused working solution should be discarded after completion of the assay. Any remaining DMSO stock can be stored for up to 6 months at -20°C.

ΔNote: PMA is a potential carcinogen. Wear gloves when using this reagent.
ΔNote: The final concentration of PMA recommended here (200 nM) can be adjusted if necessary (see Figure 3).

5.3 Dihydrorhodamine 123 Assay Reagent

1. The vial contains 50 µL of 5 mg/mL dihydrorhodamine in DMSO. Thaw at room temperature.
3. Dilute a portion of the DMSO stock solution 1:1,000 in PBS to make a 10X working solution. Any unused working solution should be discarded after completion of the assay. Any remaining DMSO stock can be stored for up to 6 months at -20°C.

5.4 Assay Buffer (basal medium not included in the kit)

1. To 500 ml of RPMI 1640 base medium (not supplied), add 5 g Bovine Serum Albumin Assay Reagent and 500 µL Calcium Chloride (1M) Assay Reagent.
2. This Assay Buffer is not intended to be sterile and does not need to be prepared or used in a tissue culture hood. As it contains no preservatives, store remaining Assay Buffer frozen at -20°C or sterile filter and store at 4°C.

6. Sample Preparation

General sample information:

6.1 Whole Blood:

1. Collect blood within 2 hours of performing the assay.
2. Use appropriate anti-coagulants (EDTA or heparin).

6.2 Other Cells:

1. Suspend the cells to be analyzed in Assay Buffer at a concentration of approximately 1 x 10^6 cells/mL.
2. Pre-warm the suspension in a water bath at 37°C for 15 minutes.

7. Assay Procedure

– Assay all standards, controls and samples in duplicate.

7.1 Cell preparation

1. Prepare cells as described in Section 6.
2. Add 100 µL of whole blood or other cells (as appropriate) to a clean polypropylene test tube.

7.2 Running the assay:

1. Add 10 µL of the 10X working stock solution of Dihydrorhodamine 123 Assay Reagent to the whole blood/cells. Incubate in a water bath at 37°C for 15 minutes.
2. Add 25 µL of the 5X Cell-Based PMA working solution, 25 µL of PBS or 25 µL of another experimental stimulus. Incubate in a water bath at 37°C for 45 minutes.
3. Add 2 mL of the RBC Lysis Buffer (1X). Incubate in a water bath at 37°C for 3-20 minutes (time should be determined empirically by the user). Centrifuge at 500 x *g* for 10 minutes at room temperature.
4. Carefully aspirate the supernatant and resuspend the cell pellet in 0.5 mL of Assay Buffer.
5. Analyze by flow cytometry. Rhodamine 123 emits a green fluorescence (~530 nm) similar to fluorescein isothiocyanate (FITC). Neutrophils typically have an intermediate forward angle light scatter and high orthogonal (side) scatter, whereas monocytes have a much higher forward angle light scatter and a lower orthogonal light scatter see Figure 1).

ΔNote: If neutrophils and monocytes are not visible by flow cytometry this may be due to poor RBC lysis in which case the incubation with RBC Lysis buffer should be performed for > 20 minutes, or the lysis should be repeated by the addition of a further 2 mL of RBC Lysis Buffer. The problem could also be due to excessive lysis resulting in the lysis of neutrophils. In this case, the lysis step should be performed for 5 minutes at room temperature.

8. FAQs / Troubleshooting

www.abcam.com/assaykitguidelines

9. Typical Data

Data provided **for demonstration purposes only.**

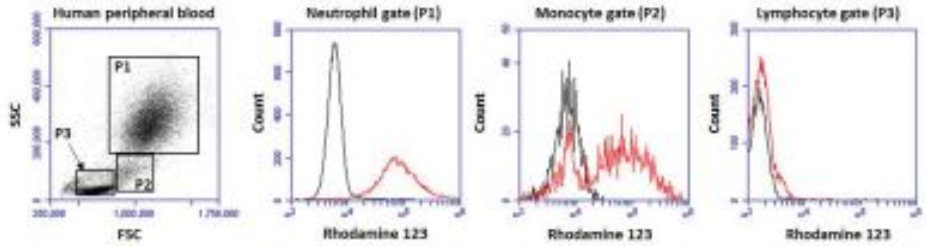


Figure 1. Flow cytometric analysis of human peripheral blood.

Human peripheral blood was treated with Dihydrorhodamine 123 Assay Reagent followed by stimulation with PMA for 45 minutes to induce the respiratory burst. The RBC were lysed and the leukocytesanalysed by flow cytometry. *Left panel:* Forwardangle light scatter(FSC) and side scatter (SSC) segregate neutrpgils (gate P1), monocytes (gate P2) and lymphocytes (gate P3) for susequent analysis in the other panels.

Remaining panels: Enhanced oxidation of Dihydrorhodamine 123 Assay Reagent to rhodamine 123 is indicated by a right shift in the x-axis FL1 fluorescence in the gated neutrophils and monocytes, but not in the lymphocytes. The black lines represent untreated cells and the red lines represent PMA-treated cells.

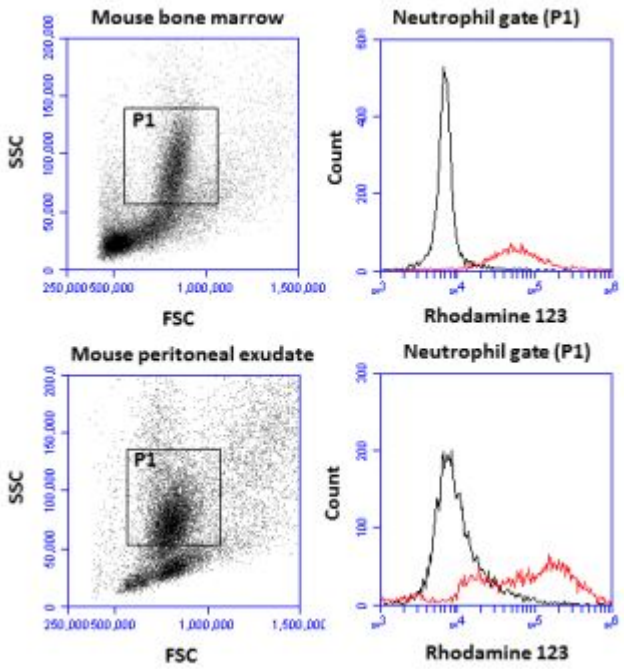


Figure 2. Flow cytometric analysis of mouse neutrophil respiratory burst.

Mouse bone marrow cells (top) and caesi-induced peritoneal exudate cells (bottom) were treated with Dihydrorhodamine 123 Assay Reagent followed by stimulation with PMA (100 nM) for 45 minutes to induce the respiratory burst. The RBC were lysed and the leukocytes analyzed by flow cytometry. *Left panels:* The neutrophils are identified by an intermediate forward angle light scatter (FSC) and high side scatter (SSC), and are gated (P1) for subsequent analysis in the other panels. *Right panels:* Enhanced oxidation of Dihydrorhodamine 123 Assay Reagent to rhodamine 123 is indicated by a right shift in the x-axis FL1 fluorescence in the gated neutrophils from samples treated with PMA (red line) versus untreated cells (black line).

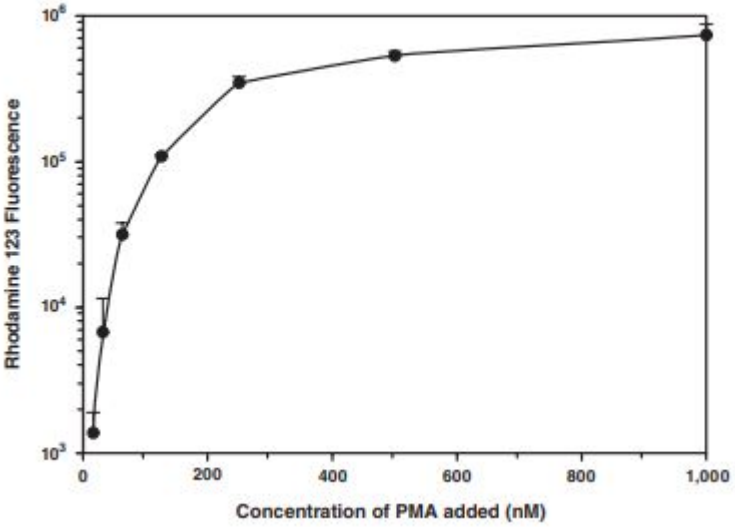


Figure 3. PMA dose-response.

Human peripheral blood was treated with Dihydrorhodamine 123 Assay Reagent followed by stimulation with the indicated concentrations of PMA for 45 minutes to induce the respiratory burst. The RBC were lysed and the leukocytes analyzed by flow cytometry.

10. Notes

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