Neutrophil/Monocyte Respiratory Burst Assay Kit

Item No. 601130

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
400088	Dihydrorhodamine 123 Assay Reagent	1 vial/50 µl	-20°C
400145	PMA (1 mM) Assay Reagent	1 vial/50 µl	-20°C
601077	RBC Lysis Buffer (10X)	1 vial/10 ml	4°C
400086	Bovine Serum Albumin Assay Reagent	1 vial/5 g	4°C
400087	Calcium Chloride (1 M) Assay Reagent	1 vial/1 ml	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section on page 3 and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. RPMI 1640 cell culture medium
2. A source of human or animal blood or leukocytes
3. Phosphate Buffered Saline (PBS)
4. Swinging-bucket tabletop centrifuge (*e.g.*, Sorvall® RT-6)
5. 12 x 75 mm polypropylene test tubes
6. Flow cytometer

INTRODUCTION

About This Assay

Cayman's Neutrophil/Monocyte Respiratory Burst Assay Kit enables researchers interested in innate immune function to induce and quantify a respiratory burst response in neutrophils and monocytes by flow cytometry. The Dihydrorhodamine 123 (DHR 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.[1-5] The assay has been validated in human and mouse whole blood, or using other sources of leukocytes. The assay reagents are not species-specific, and are expected to function in any species or cell type capable of producing a NADPH oxidase-dependent respiratory burst response.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Assay Buffer *(basal medium not included in kit)***

   To 500 ml of RPMI 1640 base medium (not provided), add 5 g Bovine Serum Albumin (BSA) Assay Reagent (Item No.400086) and 500 µl of 1M Calcium Chloride(1 M) Assay Reagent (Item No. 400087). 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.

2. **Dihydrorhodamine 123 Assay Reagent - (Item No. 400088)**

   The vial contains 50 µl of 5 mg/ml DHR 123 in DMSO. Warm to room temperature until thawed. 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 up to six months at -20°C.

3. **PMA (1 mM) Assay Reagent - (Item No. 400145)**

   The vial contains 50 µl of 1 mM PMA in DMSO. Warm to room temperature until thawed. 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 up to six months at -20°C. PMA is a potential carcinogen. Wear gloves when using this reagent. The final concentration of PMA recommended here (200 nM) can be adjusted as necessary (see Figure 3).

4. **Red Blood Cell Lysis Buffer**

   On the day of use, combine 1 ml of RBC Lysis Buffer (10X) (Item No. 601077) with 9 ml of distilled water. Warm to room temperature prior to use. Discard the combined reagent after 48 hours. The remaining unused RBC Lysis Buffer (10X) can be stored at 4°C for one year.

ASSAY PROTOCOL

Performing the Assay

1. Whole blood: Collect blood within two hours of performing the assay. Use appropriate anticoagulants (EDTA or heparin). Add 100 µl of whole blood to a clean polypropylene test tube.

   Other cells: Suspend the cells to be analyzed in Assay Buffer at a concentration of ~1.0 x 10^6 cells/ml. Pre-warm the cell suspension for 15 minutes in a 37°C water bath. Add 100 µl of the cell suspension to a clean polypropylene test tube.
2. Add 10 µl of the 10X working stock of DHR 123. Incubate in a 37°C water bath for 15 minutes.
3. Add 25 µl of the 5X PMA working stock, or 25 µl of PBS, or 25 µl of another experimental stimulus. Incubate in a 37°C water bath for 45 minutes.
4. Add 2 ml of the Red Blood Cell Lysis Buffer. Incubate in a 37°C water bath for 3-20 minutes (time should be determined empirically by user). Centrifuge for 10 minutes at room temperature at 500 x g.
5. Carefully aspirate supernatant and resuspend the cell pellet in 0.5 ml Assay Buffer.
6. 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 higher forward angle light scatter and a lower orthogonal light scatter (see Figure 1 on page 10).

Performance Characteristics

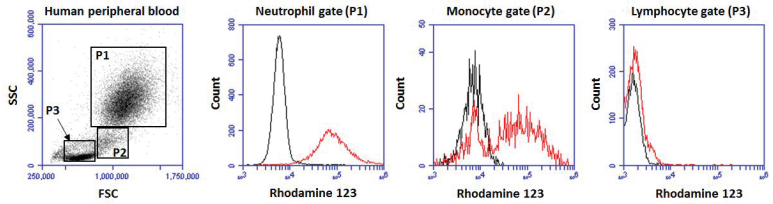

Figure 1. Flow cytometric analysis of human peripheral blood. Human peripheral blood was treated with DHR 123 followed by stimulation with PMA for 45 minutes to induce the respiratory burst. The RBC were lysed and the leukocytes analyzed by flow cytometry. *Left panel:* Forward angle light scatter (FSC) and side scatter (SSC) segregate neutrophils (gate P1), monocytes (gate P2), and lymphocytes (gate P3) for subsequent analysis in the other panels. *Remaining panels:* Enhanced oxidation of DHR 123 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.

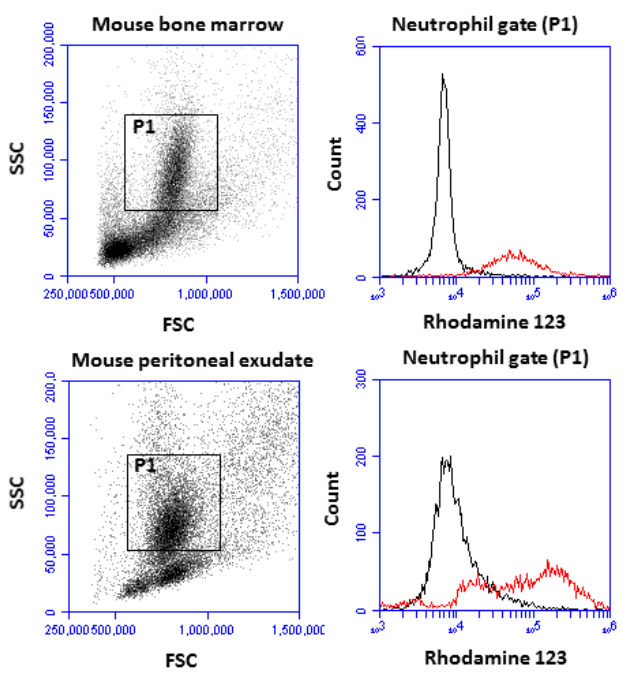

Figure 2. Flow cytometric analysis of mouse neutrophil respiratory burst. Mouse bone marrow cells (top) and casein-induced peritoneal exudate cells (bottom) were treated with DHR 123 followed by stimulation with PMA (100 nM) for 45 minutes to induce the respiratory burst. The RBC were lysed and the leukocytes 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 DHR 123 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).

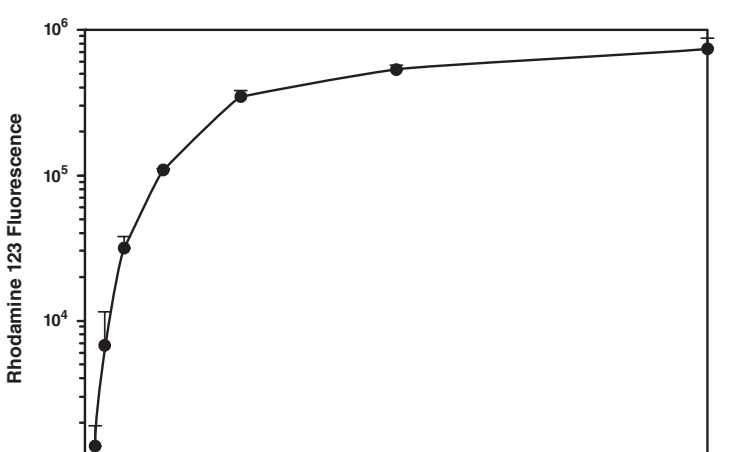

Figure 3. PMA dose-response. Human peripheral blood was treated with DHR 123 followed by stimulation with the indicated concentrations of PMA for 45 minutes to induce the respiratory burst. The RBC were lysed and the leukocytes analyzed by flow cytometry.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Neutrophils and monocytes are not identifiable by flow cytometry	A. Poor RBC lysis resulted in excessive number of RBC in prep B. Excessive RBC lysis resulted in lysis of neutrophils	A. Transfer the tube to a 37°C water bath for >20 minutes or lyse again with 2 ml more RBC lysis buffer B. Lyse RBCs for 5 minutes at room temperature
Poor RBC lysis	Insufficient time or temperature for lysis to occur	See above

References

1. Lieberman, M.M., Sachanandani, D.M., and Pinney, C.A. Comparative study of neutrophil activation by chemiluminescence and flow cytometry. *Clin. Diagn. Lab. Immunol.* **3(6)**, 654-662 (1996).
2. O'Gorman, M.R.G. and Corrochano, V. Rapid whole-blood flow cytometry assay for diagnosis of chronic granulomatous disease. *Clin. Diagn. Lab. Immunol.* **2(2)**, 227-232 (1995).
3. Richardson, M.P., Ayliffe, M.J., Helbert, M., *et al.* A simple flow cytometry assay using dihydrorhodamine for the measurement of the neutrophil respiratory burst in whole blood: Comparison with the quantitative nitrobluetetrazolium test. *J. Immunol. Methods* **219(1-2)**, 187-193 (1998).
4. Avendaño, A., Sales-Pardo, I., Marin, L., *et al.* Oxidative burst assessment and neutrophil-platelet complexes in unlysed whole blood. *J. Immunol. Methods* **339(2)**, 124-131 (2014).
5. Kooy, N.W., Royall, J.A., Ischiopoulos, H., *et al.* Peroxynitrite-mediated oxidation of dihydrorhodamine 123. *Free Radic. Biol. Med.* **16(2)**, 149-156 (1994).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.