Intracellular O_2 Respiratory Burst Imaging Kit

Item No. 601020

Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. For best results, remove components and store as stated below.

Item Number	Item	100 Tests Quantity/Size	Storage
601021	MitoImage™ $NanO_2$ Assay Reagent	1 vial/lyophilized powder	4°C
10009322	Cell-Based Assay Buffer Tablet	1 vial/1 tablet	Room Temperature
600332	Cell-Based Assay Hoechst Dye	1 vial/50 µl	-20°C
600802	Cell-Based Assay Glucose Oxidase	1 vial/2 mg	-20°C

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 laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

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. When handling the solution or stained samples, it is recommended that they be shielded from light.

Materials Needed But Not Supplied

1. A 96-well plate for culturing cells.
2. A microscope equipped with a UV filter set to visualize Hoechst staining of nuclei at excitation and emission at 350 nm and 461 nm, respectively. For the MitoImage™ $NanO_2$ signal, optimal excitation is 380-405 nm and emission is 630-670 nm. A filter which excites at 505 nm or 540 nm can be used but it is less efficient.
3. Distilled water.

INTRODUCTION

Background

Generation of a respiratory (or oxidative) burst by neutrophils and macrophages is an essential component of the innate immune response. Central to the respiratory burst is the conversion of molecular oxygen (O_2) to reactive oxygen and nitrogen species, which are involved in the killing of pathogens. In this context, O_2 is consumed foremost by NADPH oxidase, which produces superoxide. Coincident with infection, additional intracellular oxygen may be utilized by numerous other enzymes, including nitric oxide synthase, lipoxygenases, and cyclooxygenases. The Intracellular O_2 Respiratory Burst Imaging Kit facilitates the visualization of intracellular oxygen depletion resulting from this critical process. As well as investigating agents that induce and modulate this process, the kit can also be used to study the various immune-related disorders associated with dysregulated NADPH oxidase activity.[1,2]

These measurements are enabled by MitoImage™ $NanO_2$, a phosphorescent probe developed by Luxcel Biosciences to monitor O_2 levels inside live eukaryotic cells by fluorescence imaging.[3] The probe is cell permeable and can be taken up and retained by cells. Once inside cells, probe phosphorescence is quenched by O_2 in a non-chemical, reversible manner; thus, the fluorescent signal is inversely proportional to intracellular O_2 levels. The use of MitoImage™ $NanO_2$ provides a fast method of monitoring oxygen depletion without the technical challenges and throughput limitations associated with micro-needles.

About This Assay

Cayman's Intracellular O_2 Respiratory Burst Imaging Kit employs MitoImage™ $NanO_2$ for detecting O_2 in living cells, as well as Hoechst Dye for counterstaining of nuclei. The assay is performed in an unsealed environment where the back diffusion of ambient oxygen is not restricted, and in this way differs from Cayman's Oxygen Consumption Rate Assay Kit (MitoXpress®-Xtra HS Method) (Item No. 600800), which is performed under a sealed environment in which the exchange of O_2 is limited. This kit is intended to be used for detecting oxygen depletion resulting from an induced respiratory burst.

PRE-ASSAY PREPARATION

Reagent Preparation

1. Preparing the MitoImage™ $NanO_2$ Staining Solution

Reconstitute the contents in the vial (Item No. 601021) with 100 µl of sterile distilled water. Mix well. Prepare a Staining Solution by diluting the Stock Solution 1:100 in the culture medium you are using for your cells. For example, if you are making 10 ml of MitoImage™ $NanO_2$ Staining Solution, add 100 µl of the MitoImage™ $NanO_2$ Stock Solution to 10 ml of culture medium. Sufficient MitoImage™ $NanO_2$ is provided to make up to 10 mls of Staining Solution. The MitoImage™ $NanO_2$ Staining Solution should be prepared just before use. The reconstituted Staining Solution is stable for up to four weeks if stored at 4°C.

2. Assay Buffer Preparation

Dissolve one Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

3. Cell-Based Assay Hoechst Dye Staining Solution Preparation

To make a Hoechst Dye staining solution, add 2 µl of Hoechst Dye to 1 ml of the culture medium used in your experiment. Mix well. The Staining Solution should be prepared just before use.

4. Glucose Oxidase Stock Solution Preparation

The Cell-Based Assay Glucose Oxidase is included in the kit to be used as an optional positive control. Addition of the reagent at the recommended concentration, in the presence of glucose, will give a rapid increase in probe signal. Prior to use, reconstitute the contents in the vial (Item No. 600802) with 0.2 ml of distilled water. The reconstituted stock solution will be stable for two months when stored at -20°C.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical experimental plate will include wells without cells, wells with cells treated with experimental compounds, and wells of untreated cells. We recommend that each treatment be performed in triplicate and that you record the contents of each well on the template sheet provided (see page 15).

Performing the Assay

1. Seed cells in a 96-well plate at a density of 10^4-10^5cells/well in 100 µl of culture medium. Culture the cells in a CO_2 incubator at 37°C for 24 hours.
2. Aspirate the supernatant. Add 100 µl of MitoImage™ NanO_2 Staining Solution to each well and incubate the cells for three hours to overnight. *NOTE: For cells in suspension, centrifuge the plate at 1,000 rpm for five minutes before aspirating the supernatant.*
3. Aspirate the supernatant and wash the cells two times with the culture medium. *NOTE: For cells in suspension, centrifuge the plate at 1,000 rpm, for five minutes before aspirating the supernatant.*
4. Add 100 µl of culture medium with or without test compounds (each sample should be run in duplicate or triplicate) and incubate the cells in a CO_2 incubator at 37°C for 24-48 hours, or for a period of time according to your experimental protocol. If you are using the included Cell-Based Assay Glucose Oxidase as a positive control, have two additional wells of cells treated with vehicle and assign them as positive control wells.
5. At the end of the experiment, centrifuge the plate for five minutes at 1,000 rpm. Aspirate the supernatant.
6. Add 100 µl of Hoechst Dye Staining Solution prepared above to each well and incubate samples at 37°C for two to five minutes.
7. Centrifuge the plate for five minutes at 1,000 rpm. Aspirate the supernatant.
8. Add 90 µl of culture medium and 10 µl of Glucose Oxidase Stock Solution to both positive control wells. Alternatively, add 90 µl of the Assay Buffer containing 100 mM glucose and 10 µl of Glucose Oxidase Stock Solution to both positive control wells.
9. Add 100 µl of Assay Buffer to each well except the postive control wells. Visualize the cells under a microscope capable of detecting fluorescence at an excitation wavelength of 390-405 nm and an emission wavelength of 640-670 nm. A filter which excites at 505 or 540 nm can be used but it is less efficient. Hoechst Dye staining of cell nuclei can be visualized with a UV filter set.

PERFORMANCE CHARACTERISTICS

NOTE: This kit uses MitoImage™ $NanO_2$ to detect changes in intracellular oxygen levels in response to respiratory burst. For analysis of changes in oxygen consumption or respiration rate, we recommend using Cayman's Oxygen Consumption Rate Assay Kit (MitoXpress® - Xtra HS Method, Item No. 600800).

Representative Staining Results

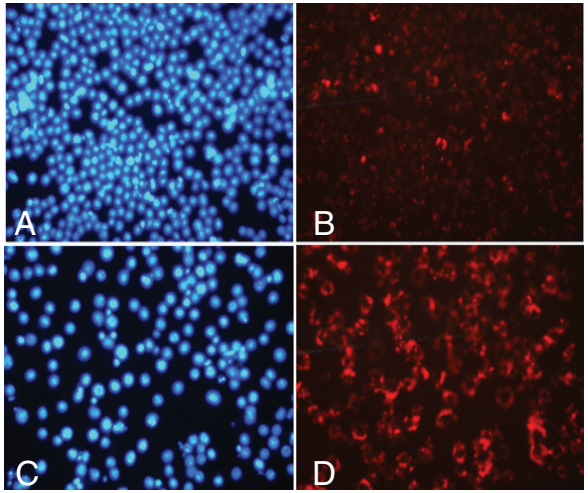


Figure 1. LPS significantly decreases intracellular O_2 levels in RAW 264.7 cells, indicated by an increase in $NanO_2$ signal. RAW264.7 cells were seeded in a 96-well plate at a density of 20,000 cells/well in 100 µl culture medium and incubated in a cell culture incubator overnight. The next day, cells were labeled with MitoImage™ $NanO_2$, as described in the booklet. Cells were then cultured in 100 µl culture medium containing either vehicle (*upper panels*) or 1 µg/ml LPS (*lower panels*) for 48 hours. Left panels show staining of nuclei by Hoechst Dye. Panels on the right show fluorescence signal from MitoImage™ $NanO_2$ (signal inversely proportional to O_2 concentration) in fields corresponding to those in left panels.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
High fluorescence signal in all wells including vehicle controls	A. Overgrowth of cells B. Culture medium containing phenol red not completely removed	A. Use lower cell density B. Wash the cells with the Assay Buffer
Low or no fluorescence signal	Cells are not healthy	Use healthy cells
Decreased fluorescence in control well	Photobleaching of probe	A. Reducing lamp intensity B. Reducing illustration time/ frequency

References

1. Dinauer, M.C. and Orkin, S.H. Chronic granulomatous disease. Molecular genetics. *Hematol. Oncol. Clin. North. Am.* **2(2)**, 225-240 (1988).
2. Moraes, T.J., Zurawska, J.H., and Downey, G.P. Neutrophil granule contents in the pathogenesis of lung injury. *Curr. Opin. Hematol.* **13(1)**, 21-27 (2006).
3. Dmitriev, R.I., Zhdanov, A.V., Nolan, Y.M., *et al.* Imaging of neurosphere oxygenation with phosphorescent probes. *Biomaterials* **34(37)**, 9307-9317 (2013).

Related Products

Hydrogen Peroxide Cell-Based Assay Kit - Item No. 600050
Nitric Oxide Cell-Based Assay Kit - Item No. 10009419
Oxygen Consumption/Glycolysis Dual Assay Kit - Item No. 601060
Oxygen Consumption/MitoMembrane Potential Dual Assay Kit - Item No. 600880
Oxygen Consumption Rate Assay Kit (MitoXpress®-Xtra HS Method) - Item No. 600800

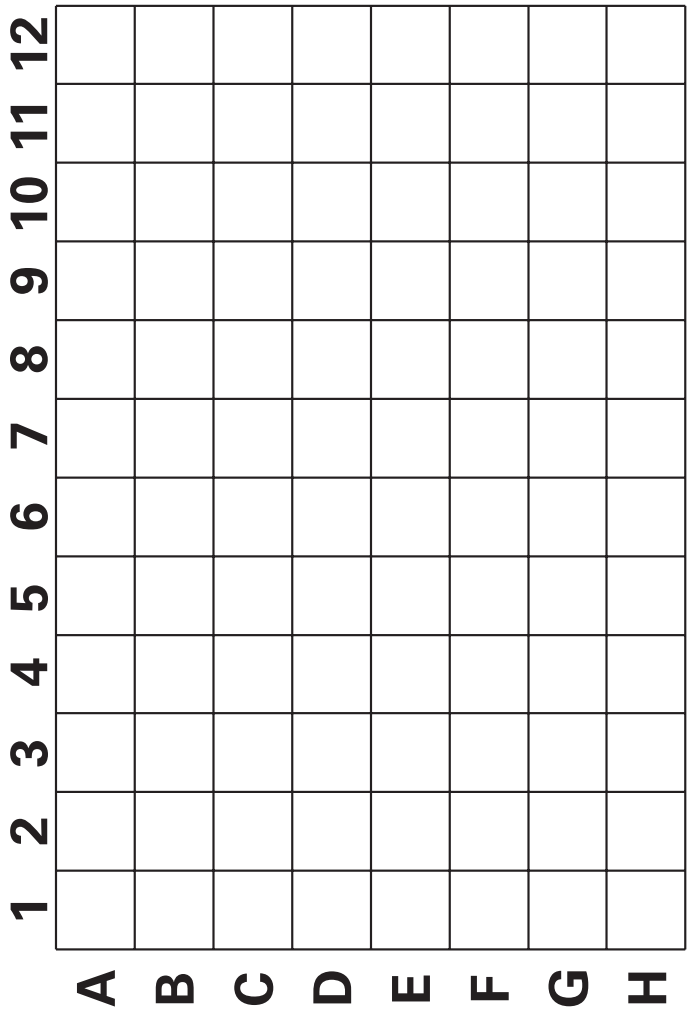
Warranty and Limitation of Remedy

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material **will meet our specifications at the time of delivery**. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a **refund** of the purchase price, or at Cayman's option, the **replacement**, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



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