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ab219932
Nitric Oxide Assay Kit (Fluorometric - Orange)

For the rapid, sensitive and accurate measurement of NO in live cells

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

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1. Overview

Nitric Oxide Assay Kit (Fluorometric - Orange) (ab219932) is a sensitive fluorometric assay to monitor intracellular nitric oxide (NO) levels in live cells. The assay uses an orange dye that can react with NO to generate a bright orange fluorescent product that can be easily detected at Ex/Em = 540/590 nm, using the same filter set as Cy3® or TRITC.

The assay can be detected by fluorescence microscopy, microplate fluorometry or high-content imaging. It can be easily adapted to use in 384-well microplate format.

Nitric oxide (NO) is an important biological regulator involved in numbers of physiological and pathological processes. Altered NO production is implicated in various immunological, cardiovascular, neurodegenerative and inflammatory diseases. As a free radical, NO is rapidly oxidized and there is relatively low concentrations of NO existing in vivo. It has been challenging to detect and understand the role of NO in biological systems.

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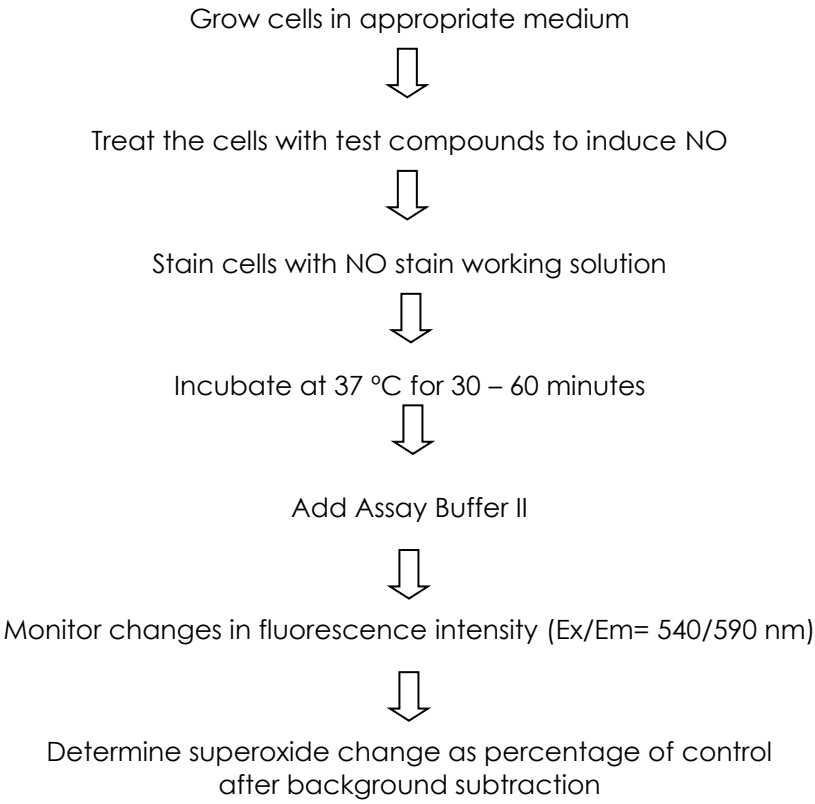
2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website www.abcam.com.

4. Storage and Stability

Store Kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

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

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
500X NO Orange Dye	50 µL	-20°C	-20°C
Assay Buffer I	20 mL	-20°C	-20°C
Assay Buffer II	20 mL	-20°C	-20°C

7. Materials Required, Not Supplied

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

- Fluorometric microplate reader (bottom read mode) or fluorescence microscopy capable of measuring fluorescence at Ex/Em = 520/605 nm
- PBS or HHBS buffer
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- General tissue culture supplies
- Sterile 96 well plate with clear flat bottom, preferably black (if performing assay in microplate format). Use a poly-D-lysine coated plate for suspension cells

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 500X NO Orange Dye:

Ready to use as supplied. Equilibrate to room temperature before use.

Δ Note: 20 µL of 500X NO Orange Dye stock solution is enough 1 x 96 well plate.

Aliquot unused 500X NO Orange dye so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Avoid repeated freeze-thaw cycles.

9.2 Assay Buffer I:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 Assay Buffer II:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

10. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- Each cell line should be evaluated on an individual basis to determine the optimal cell density.
- The protocol described here is for 96-well microplate format. You can adapt protocol for larger or culture vessels by scaling up volumes accordingly.

10.1 Grow cells:

10.1.1 Adherent cells: plate cells overnight in growth medium at 3-8 x 10^4 cells/90 µL per well.

Δ Note: for 384 well plate, use 8-20 x 10^3/20 µL per well.

10.1.2 Suspension cells: on the day of the assay, centrifuge cells from the culture medium and resuspend the cell pellet in culture medium at 1.25-2.5 x 10^5 cells/90 µL per cell in a poly-D-lysine coated plate.

Δ Note: for 384 well plate, use 3-6 x 10^4 cells/20 µL per well.

10.1.3 Centrifuge plate at 800 rpm for 2 minutes with brake off.

10.2 Prepare NO stain working solution:

10.2.1 Add 20 µL of 500X NO Orange Dye stock solution (Step 9.1) into 10 mL of Assay Buffer I (Step 9.1) and mix well.

Δ Note: NO stain working solution is stable for at least 2 hours at room temperature.

10.3 Run the NO assay:

10.3.1 Induce NO formation by treating cells with 10 µL of 10X test compounds in PBS or HHBS. For untreated cells, add 10 µL of compound buffer.

Δ Note: for 384 well plate, add 5 µL of 5X test compounds.

Δ Note: Do not remove the test compounds

Suggested positive controls:

- NONOate positive control: HeLa cells treated with 1 mM DEA/NONOate at 37°C for 30 minutes (exogenous NO formation)

– RAW 264.7 cells treated with 20 µg/mL of lipopolysaccharide (LPS) and 1 mM L-arginine at 37°C for 16 hours (endogenous NO formation)

Δ Note: it is not necessary to wash cells before adding compound. However, if tested compounds are serum sensitive, growth medium and serum factors can be aspirated away before adding compounds. In that is the case, add 90 µL/well of 1X HHBS or the buffer of your choice after aspiration. Alternatively, cells can be grown in serum-free media.

10.3.2 Add 100 µL/well of NO stain Working Solution into the cell plate.

Δ Note: Do not remove the test compounds

Δ Note: for 384 well plate, add 25 µL/well.

10.3.3 Incubate cell plate at 37°C for 30-60 minutes, or required period of time, protected from light.

10.3.4 Remove stain solution from cells.

10.3.5 Add 100 µL/well of Assay Buffer II (Step 9.3). DO not wash cells before adding Assay Buffer II.

Δ Note: for 384 well plate, add 25 µL Assay Buffer II/well.

10.3.6 Monitor fluorescence increase at Ex/Em = 540/590 nm (cut off 570 nm) with bottom read mode. Alternatively, monitor the fluorescence signal using a fluorescence increase with a TRITC filter set.

11. Data Analysis

FOR MICROPLATE READER

- Subtract blank reading (assay buffer only) from all measurements (control and treated).
- Using fluorescence intensity, determine fold change between control and treated cells.

FOR FLUORESCENCE MICROSCOPY

- We recommend acquiring several images per well.
- We recommend data analysis after coding and mixing images to ensure unbiased results.
- For manual analysis, if you do not have a specific software installed in your microscope, you can download ImageJ, an open source image processing designed for scientific multidimensional images by the National Institute of Health (NIH).

12. Typical Data

Data provided for **demonstration purposes** only.

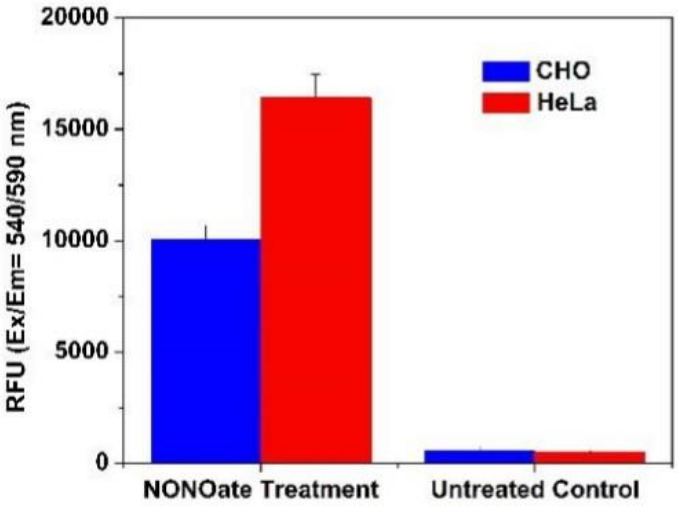


Figure 1. Exogenous nitric oxide (NO) production upon DEA/NONOate treatment (NO donor). CHO-K1 (blue) and HeLa (red) cells were incubated with NO stain working solution at 37°C for 30 minutes, and then removed to stop the staining. Cells were further treated with or without 1mM DEA/NONOate in HBSS buffer (with 10 mM HEPES (pH=6.2)) at 37°C for 30 minutes. The solution in each well was removed and Assay Buffer II was added before measuring fluorescence. The fluorescence signal was monitored at Ex/Em = 540/590 nm (cut off = 570 nm) with bottom read mode using a FlexStation microplate reader (Molecular Devices).

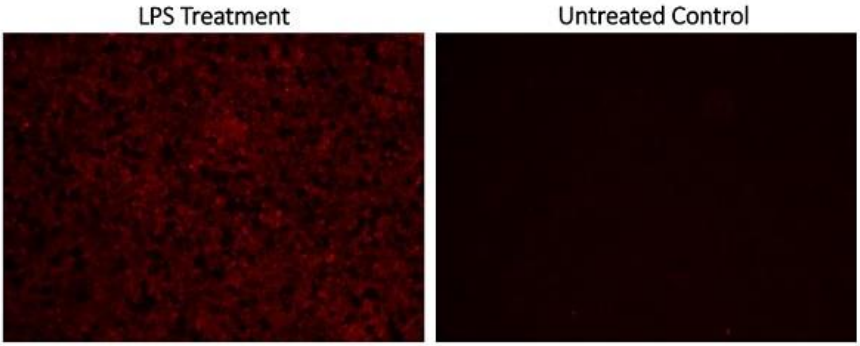


Figure 2. Endogenous nitric oxide (NO) production in RAW 264.7 macrophage cells. Raw 264.7 cells were seeded overnight (10^5 cells/well/100 µL) in a black wall/clear bottom 96-well plate. Left: cells were treated with 20 µg/mL of lipopolysaccharide (LPS) and 1 mM L-Arginine (L-Arg) and co-incubated with 0.5X NO stain working solution at at 37°C for 16 hours. Right: control RAW 264.7 cells were left untreated in cell culture medium and co-incubted with 0.5X NO stain working solution at 37°C for 16 hours. The solution in each well was removed and Assay Buffer II was added before fluorescence measurement. The fluorescence signal was measured using fluorescence microscope with a TRITC filter.

13. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)