Version 2a Last updated 30 January 2019

ab219934
Nitric Oxide Assay Kit (Flow cytometry - Red)

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.

Table of Contents

1. Overview

Nitric Oxide Assay Kit (Flow cytometry - Red) (ab219934) is a sensitive fluorometric assay to monitor intracellular nitric oxide (NO) levels in live cells using a flow cytometer. The assay uses a red dye that can react with NO to generate a bright red fluorescent product that can be easily detected at Ex/Em = 630/660 nm, using the same filter set as Texas Red®.

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.

Texas Red® is trademark of Life Technologies.

2. Protocol Summary

Grow cells in appropriate medium

Stain cells with NO stain working solution

Incubate cells with test compounds at 37°C

Analyze cells with a flow cytometer
(Ex/Em=630/660 nm)

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.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- 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.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -80°C 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 Red Dye	100 µL	-80°C	-80°C
Assay Buffer	10 mL	-80°C	-80°C
NONOate Positive Control	1 vial	-80°C	-80°C

7. Materials Required, Not Supplied

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

- Flow cytometer equipped with adequate filters to measure fluorescence at Ex/Em = 630/660 nm (FL4 channel)
- Double distilled water (ddH_2O)
- 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
- 12 x 75 mm tubes for flow cytometry
- 0.5 mM EDTA (for adherent 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 Red Dye:

before use. Aliquot unused 500X NO Red dye so that you have enough volume to perform the desired number of assays. Store at -80°C protected from light. Avoid repeated freeze-thaw cycles.

9.2 Assay Buffer (10 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot so that you have enough reagent to perform the desired number of assays. Keep on ice while in use. Store at -80°C.

9.3 NONOate Positive Control (lyophilized):

Add 200 µL of ddH_2O into the vial to make 50 mM stock solution. Aliquot so that you have enough volume to perform the desired number of assays, Keep on ice while in use. Store at -80°C protected from light.

10. Assay Procedure – flow cytometry

- 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.

10.1 Grow cells:

10.1.1 Suspension cells: prepare cells in 0.5 mL warm culture medium at a density of 5 x 10^5 – 10^6 cells/mL.

10.1.2 Adherent cells: gently lift the cells with 0.5 mM EDTA to keep the cells intact. Wash cells once with cell culture medium and resuspend cells in 0.5 mL warm culture medium at a density of 5 x 10^5 – 10^6 cells/mL.

10.2 Run NO assay:

10.2.1 Add 1 µL 500X NO Red Dye stock solution into 0.5 mL cell suspension.

10.2.2 Induce NO formation by treating stained cells with test compounds and incubating them in a 37°C/5% CO_2 incubator for the desired period of time. For untreated cells, add 10 µL of the same buffer used to dilute the test compounds.

Δ Note: for example, incubate RAW 264.7 cells with 20 µg/mL of lipopolysaccharide (LPS) and 1 mM L-arginine at 37°C for 16 hours to induce endogenous NO formation/

10.2.3 NONOate positive control treatment (exogenous NO formation):

10.2.3.1 Spin down cells that have been pre-incubated with 500X NO Red dye for 30 minutes.

10.2.3.2 Dilute the 50mM positive control stock solution (Step 9.3) with Assay Buffer to make 1 mM NONOate positive control working solution

10.2.3.3 Resuspend cells in the positive control working solution and incubate at 37°C for another 30 minutes.

10.2.4 Monitor fluorescence increase with a flow cytometer at Ex/Em = 630/660 nm (FL4 channel).

11. Data Analysis

The following are general guidelines. Specific methods of analysis will vary with different flow cytometer analysis programs.

- Establish appropriate FSC vs SSC gates to exclude debris and cell aggregates.
- Using mean fluorescent intensity, determine fold change between control and treated cells.

12. Typical Data

Data provided for **demonstration purposes** only.

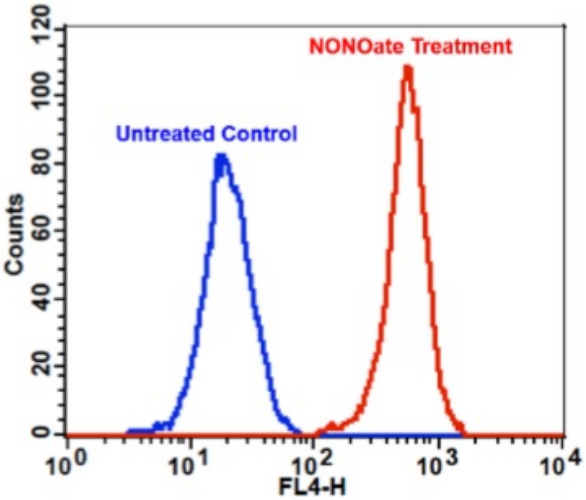

Figure 1. Exogenous nitric oxide (NO) production in Jurkat cells upon DEA/NONOate treatment (NO donor). Cells were incubated with the NO Red Dye in a 37°C/5% CO_2 incubator for 30 minutes, followed by a wash in HHBS buffer. Cells were then left untreated (blue) or further treated with 1 mM DEA/NONOate (red) in Assay Buffer at 37°C for an additional 30 minutes. Fluorescent intensitites were measured with a FACScalibur flow cytometer (BD Biosciences) using FL4 channel.

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abcam.com | +64-(0)9-909-7829