

Cell Meter™ Fluorimetric Intracellular Nitric Oxide (NO) Activity Assay Kit *Orange Fluorescence Optimized for Flow Cytometry*

Catalog number: 16351

Unit size: 100 Tests

Component	Storage	Amount
Component A: 500X Nitrixyte™ Orange	Freeze (<-15 °C), Avoid Light	100 µL
Component B: NONOate Positive Control	Freeze (<-15 °C), Dessicated, Avoid Light	1 vial (lyophilized powder)
Component C: Assay Buffer	Freeze (<-15 °C), Avoid Light	1 bottle (10 mL)

OVERVIEW

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. Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit provides a sensitive tool to monitor intracellular NO level in live cells. Nitrixyte™ probes are developed and used in our kit as an excellent replacement for DAF-2 for the detection and imaging of free NO in cells. Compared to the commonly used DAF-2 probe, Nitrixyte™ probes have better photostability and enhanced cell permeability. This particular kit uses Nitrixyte™ Orange that can react with NO to generate a bright orange fluorescent product that has spectral properties similar to Cy3® and TRITC. Nitrixyte™ Orange can be readily loaded into live cells, and its fluorescence signal can be conveniently monitored using the filter set of Cy3® or TRITC. This kit is optimized for flow cytometry applications.

AT A GLANCE

Protocol summary

1. Prepare cells (0.5 - 1 × 10⁶ cells/mL)
2. Add 1 µL 500X Nitrixyte™ Orange into 0.5 mL cell suspension
3. Incubate cells with test compounds and Nitrixyte™ Orange at 37°C
4. Analyze with a flow cytometer

Important Thaw all the components at room temperature before use.

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm
Emission:	590 nm
Instrument specification(s):	FL2 channel or PE channel

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. NONOate Positive Control stock solution (50 mM):

Add 200 µL of ddH₂O into the vial of NONOate Positive Control (Component B) to make 50 mM stock solution.

PREPARATION OF WORKING SOLUTION

1. NONOate Positive Control working solution

Dilute the NONOate Positive Control stock solution to a 1 - 2 mM working solution with Assay Buffer (Component C).

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit

<https://www.aatbio.com/resources/guides/cell-sample-preparation.html>

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 1 µL of 500X Nitrixyte™ Orange (Component A) into 0.5 mL cell suspension.

Note For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact, and wash the cells once with serum-containing media prior to incubation with Nitrixyte™ Orange.

2. Incubate cells with test compounds and Nitrixyte™ Orange at 37°C for a desired period of time to generate endogenous or exogenous NO.

Note The appropriate incubation time depends on the individual cell type and test compound used. Optimize the incubation time for each experiment.

Note We have used Raw 264.7 cells incubated with 1X Nitrixyte™ Orange, 20 µg/mL of lipopolysaccharide (LPS) and 1 mM L-Arginine (L-Arg) in cell culture medium at 37°C for 16 hours.

3. Spin down cells that have pre-incubated with Nitrixyte™ Orange for 30 minutes. Resuspend cells with 1 mM DEA NONOate positive control working solution, and incubate at 37°C for another 30 minutes. See Figure 1 for details.
4. Monitor the fluorescence intensity at the FL2 channel (Ex/Em = 488/590 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.

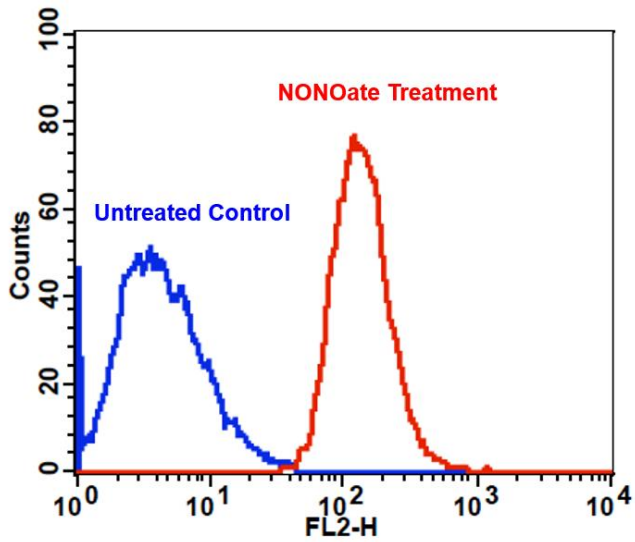


Figure 1. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat#16351). Cells were incubated with Nitrixyte™ Orange at 37 °C for 30 minutes. Cells were further treated with (Red line) or without (Blue line) 1 mM DEA NONOate in Assay Buffer (Component C) at 37 °C for an additional 30 minutes. The fluorescence signal was monitored at FL2 channel using a flow cytometer (BD FACSCalibur).

DISCLAIMER

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