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

Catalog number: 16356
Unit size: 100 Tests

Component	Storage	Amount
Component A: 500X Nitrixyte™ Red	Freeze (<-15 °C), Minimize light exposure	100 µL
Component B: NONOate Positive Control	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder)
Component C: Assay Buffer	Freeze (<-15 °C)	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™ Red that can react with NO to generate a bright red fluorescent product that has spectral properties similar to Texas Red®. Nitrixyte™ Red can be readily loaded into live cells, and its fluorescence signal can be conveniently monitored using the filter set of Red. This kit is optimized for flow cytometry applications.

AT A GLANCE

Protocol summary

1. Prepare cells (0.5 - 1×10^6 cells/mL)
2. Add 1 µL 500X Nitrixyte™ Red
3. Incubate cells with test compounds and Nitrixyte™ Red at 37 ºC for desired period of time
4. Analyze cells with a flow cytometer using FL4 channel

Important
Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	640 nm laser
Emission:	660/20 nm filter
Instrument specification(s):	APC 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 treatment stock solution (50 mM):
Add 200 uL of ddH2O into the vial of NONOate Positive Control (Component B) to make 50 mM NONOacte Positive Control treatment stock solution.

PREPARATION OF WORKING SOLUTION

Dilute 50 mM NONOate Positive Control treatment stock solution with Assay Buffer (Component C) to make 1-2 mM NONOate positive control working solution.

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. For each sample, prepare cells in 0.5 mL warm medium or buffer of your choice at a density of 5×10^5 to 1×10^6 cells/mL.

   Note Each cell line should be evaluated on an individual basis to determine the optimal cell density for NO induction.

2. Add 1 µL of 500X Nitrixyte™ Red (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™ Red.

3. Incubate cells with test compounds and Nitrixyte™ Red at 37 ºC 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 Nitrixyte™ Red working solution, 20 µg/mL of lipopolysaccharide (LPS) and 1 mM L-Arginine (L-Arg) in cell culture medium at 37 ºC for 16 hours.

4. Spin down cells that have pre-incubated with Nitrixyte™ Red for 30 minutes. Resuspend cells with 1 mM DEA NONOate positive control working solution, and incubate at 37 ºC for another 30 minutes. See Figure 1 for details.

5. Monitor the fluorescence intensity at the FL4 channel (Ex/Em = 630/660 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

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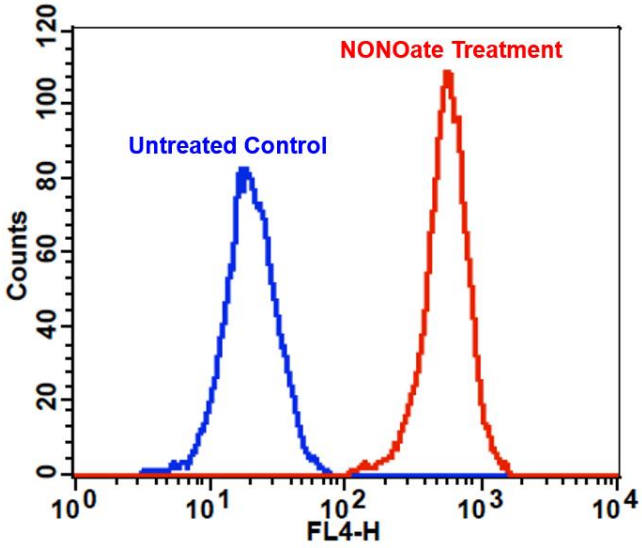

Figure 1. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat#16356). Cells were incubated with Nitrixyte™ Red at 37 °C for 30 minutes. Cells were further treated with (Red line) or without (Blue line) 1 mM DEA NONOate at 37 °C for another 30 minutes. The fluorescence signal was monitored at FL4 channel using a flow cytometer (BD FACSCalibur).