

Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit

Red Fluorescence Optimized for Flow Cytometry

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 16356 (100 assays)	Keep in freezer, avoid light	Flow Cytometer

Introduction

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 Texas Red®. This kit is optimized for flow cytometry applications.

Kit Components

Components	Amount
Component A: 500X Nitrixyte™ Red	100 µL
Component B: NONOate Positive Control	1 vial (Lyophilized powder)
Component C: Assay Buffer	1 bottle (10 mL)

Assay Protocol for Flow Cytometer

Brief Summary

Prepare cells ($0.5 - 1 \times 10^6$ cells/mL) → Add 1 µL 500X Nitrixyte™ Red → Incubate cells with test compounds and Nitrixyte™ Red at 37 °C for desired period of time → Analyze cells with a flow cytometer

Note: Thaw all the components at room temperature before use.

- 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.
- 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.
- Incubate cells with test compounds and Nitrixyte™ Red (from Step 2) at 37 °C for a desired period of time to generate endogenous or exogenous NO.
Note 1: The appropriate incubation time depends on the individual cell type and test compound used. Optimize the incubation time for each experiment.
Note 2: 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 °C for 16 hours.
- For a NONOate positive control treatment, add 200 µL of ddH₂O into the vial of Component B to make 50 mM stock solution. The stock solution is diluted to make 1-2 mM working solution with Assay Buffer (Component C). 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 °C 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.

Data Analysis

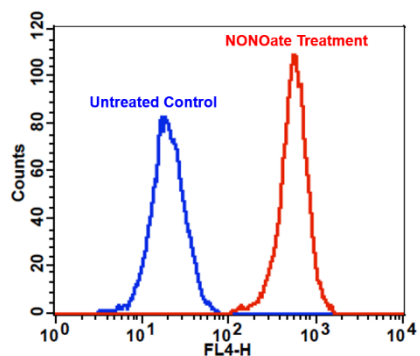


Figure 1. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Activity Assay Kit (Cat#16356). Cells were incubated with Nitrixyte™ Red at 37 °C, 5% CO₂ for 30 minutes. Spin down and wash cells with Hanks and 10 mM HEPES buffer. Cells were further treated with (Red line) or without (Blue line) 1 mM DEA NONOate in Assay Buffer (Component C) at 37 °C, 5% CO₂ incubator for an additional 30 minutes. The fluorescence signal was monitored at FL4 channel using a flow cytometer (BD FACSCalibur).

References

1. Rose MJ and Mascharak PK. (2008) Fiat Lux: selective delivery of high flux of nitric oxide (NO) to biological targets using photoactive metal nitrosyls. *Curr Opin Chem Biol*.
2. Mayer B. (2000) Nitric Oxide; Handbook of Experimental Pharmacology. Springer, Berlin.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.