Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit

Orange Fluorescence Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 16351 (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™ 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.

Kit Components

Components	**Amount**
Component A: 500X Nitrixyte™ Orange	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 × 10^6 cells/mL) → Add 1 µL 500X Nitrixyte™ Orange into 0.5 mL cell suspension → Incubate cells with test compounds and Nitrixyte™ Orange at 37 ºC → Analyze with a flow cytometer

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

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™ 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.

3. Incubate cells with test compounds and Nitrixyte™ Orange (from Step 2) at 37 ºC 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 1X Nitrixyte™ Orange, 20 µg/mL of lipopolysaccharide (LPS) and 1 mM L-Arginine (L-Arg) in cell culture medium at 37 ºC for 16 hours.

4. 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™ Orange 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 FL2 channel (Ex/Em=488/590 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.

Data Analysis

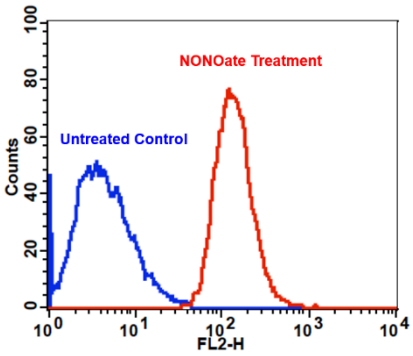

Figure 1. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat#16351). Cells were incubated with Nitrixyte™ Orange at 37 ºC, 5% CO_2 incubator for 30 minutes. Spin down and wash cells with 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 ºC, 5% CO_2 incubator for an additional 30 minutes. The fluorescence signal was monitored at FL2 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) Nitic Oxide; Handbook of Experimental Pharmacology. Springer, Berlin.