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

Catalog number: 16350
Unit size: 200 Tests

Component	Storage	Amount
Component A: 500X Nitrixyte™ Orange	Freeze (<-15 °C), Minimize light exposure	50 µL
Component B: Assay Buffer I	Freeze (<-15 °C), Minimize light exposure	1 bottle (20 mL)
Component C: Assay Buffer II	Freeze (<-15 °C), Minimize light exposure	1 bottle (20 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 fluorescence imaging and microplate reader applications.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Incubate cells with test compounds and Nitrixyte™ Orange working solution at 37°C for a desired period
3. Add Assay Buffer II
4. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 540/590 nm (Cutoff = 570 nm) or fluorescence microscope using TRITC filter

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode
Instrument:	Fluorescence microscope
Excitation:	TRITC filter
Emission:	TRITC filter
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

Add 20 µL of 500X Nitrixyte™ Orange stock solution (Component A) into 10 mL of Assay Buffer I (Component B) and mix well to make Nitrixyte™ Orange working solution. This Nitrixyte™ Orange working solution is stable for at least 2 hours at room temperature. Protect from light.

Note 20 µL of 500X Nitrixyte™ Orange stock solution is enough for one plate.

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. To stimulate endogenous NO, treat cells with 10 µL of 10X test compounds (96-well plate) or 5 µL of 5X test compounds (384-well plate) in cell culture medium or your desired buffer (such as PBS or HHBS). For control wells (untreated cells), add the corresponding amount of medium or compound buffer.

   Note It is not necessary to wash cells before adding compound. However, if tested compounds are serum sensitive, growth medium and serum factors can be aspirated away before adding compounds. Add 90 µL/well (96-well plate) and 20 µL/well (384-well plate) of 1X Hank's salt solution and 20 mM Hepes buffer (HHBS) or the buffer of your choice after aspiration. Alternatively, cells can be grown in serum-free media.

2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Nitrixyte™ Orange working solution in the cell plate. Co-incubate cells with test compound and Nitrixyte™ Orange working solution at 37°C for desired period of time, protected from light.

   Note DO NOT remove the test compounds. For a NONOate positive control treatment: Cells were incubated with Nitrixyte™ Orange working solution at 37°C for 30 minutes. The working solution was removed and cells were further incubated with 1 mM DEA/NONOate at 37°C for 30 minutes to generate nitric oxide.

   Note We have used Raw 264.7 cells incubated with 0.5X 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. See Figure 1 for details.

3. Remove solution in each well.

4. Add Assay Buffer II (Component C) 100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate.

   Note DO NOT wash cells before adding Assay Buffer II.

5. Monitor the fluorescence increase using microplate reader at Ex/Em = 540/590 nm (Cutoff = 570 nm) with bottom read mode, or take images using fluorescence microscope with a TRITC filter.

EXAMPLE DATA ANALYSIS AND FIGURES

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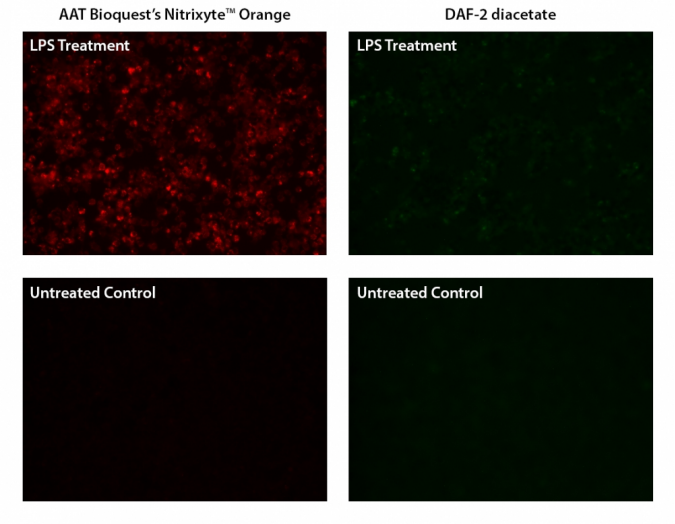

Figure 1. Fluorescence images of endogenous nitric oxide (NO) detection in RAW 264.7 macrophage. Cells were incubated with AAT's Nitrixyte™ Orange (Left) or DAF-2 diacetate (Right) at the same concentration, then treated with or without 20 µg/mL of lipopolysaccharide (LPS) and 1 mM L-arginine (L-Arg) at 37°C for 16 hours. The fluorescence signals were measured using a fluorescence microscope equipped with a TRITC (Left) or FITC (Right) filter set, respectively.

DISCLAIMER