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

Catalog number: 16359
Unit size: 200 Tests

Component	Storage	Amount
Component A: Nitrixyte™ NIR	Freeze (<-15 °C), Minimize light exposure	50 µL (500X)
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 Kits provide sensitive tools to monitor intracellular NO level in live cells. Nitrixyte™ probes are developed and used in our kits 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™ NIR that can react with NO to generate strong near-infrared (NIR) fluorescence signal. Nitrixyte™ NIR can be readily loaded into live cells, and its fluorescence signal can be conveniently monitored using the filter set of Cy5® or APC. 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™ NIR working solution
3. Add Assay Buffer II
4. Monitor fluorescence intensity at Ex/Em = 650/680 nm

Important Thaw all the kit component at room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	650 nm
Emission:	680 nm
Cutoff:	665 nm
Instrument specification(s):	Bottom read mode
Recommended plate:	Black wall/clear bottom
Instrument:	Fluorescence microscope
Excitation:	620 nm
Emission:	665 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Cy5 filter set

PREPARATION OF WORKING SOLUTION

Add 20 µL of Nitrixyte™ NIR stock solution (Component A) into 10 mL of Assay Buffer I (Component B) and mix well. The working solution is stable for at least 2 hours at room temperature.

Note 20 µL of Nitrixyte™ NIR stock solution is enough for one plate. Keep from light.

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™ NIR working solution in the cell plate. Co-incubate cells with test compound and Nitrixyte™ NIR working solution at 37°C for desired period of time, protected from light.

   Note DO NOT remove the test compounds.

   Note For a NONOate positive control treatment: Cells were incubated with Nitrixyte™ NIR 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. See Figure 1 for details. We have used Raw 264.7 cells incubated with 0.5X Nitrixyte™ NIR, 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 2 for details.

3. Remove solution in each well. 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.

4. Monitor the fluorescence increase using microplate reader at Ex/Em = 650/680 nm (cut off = 665 nm) with bottom read mode, or take images using fluorescence microscope with a Cy5® filter.

EXAMPLE DATA ANALYSIS AND FIGURES

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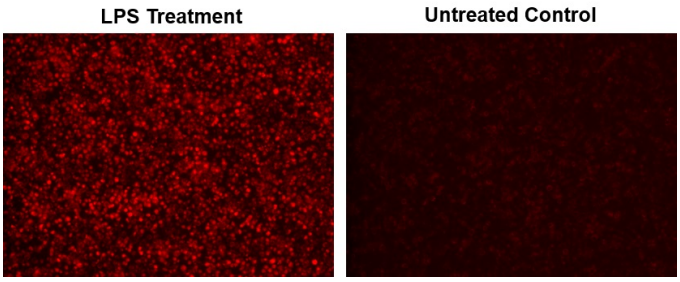

Figure 1. Fluorescence images of endogenous nitric oxide (NO) measurement in RAW 264.7 macrophage cells using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Activity Assay Kit (Cat#16359). Raw 264.7 cells at 100,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. Cells were co-incubated with Nitrixyte™ NIR, with or without 20 µg/mL of lipopolysaccharide (LPS) and 1 mM L-Arginine (L-Arg) in cell culture medium at 37 °C for 16 hours. The solution in each well was removed, and Assay Buffer II was added before fluorescence measurement. The fluorescence signal was measured using fluorescence microscope with a Cy5® filter.

DISCLAIMER