CREATIVE BIOMART®
Assay Kit

Nitric Oxide Activity Fluorimetric Assay Kit (Orange)

Product Information

Cat.No. Kit-2044

Product Overview

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 photo stability 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.

Storage

Freeze (<-15 °C), Minimize light exposure.

Size

200 Tests

Kit Components

Component A: 500X Nitrixyte Orange 1 vial (50 µL)
Component B: Assay Buffer I 1 bottle (20 mL)
Component C: Assay Buffer II 1 bottle (20 mL)

Preparation

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.

Assay Protocol

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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

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

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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.
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.