abcam

Product datasheet

Nitric Oxide Assay Kit (Fluorometric - Orange) ab219932

2 References 2 Images

Overview

Product name	Nitric Oxide Assay Kit (Fluorometric - Orange)
Detection method	Fluorescent
Sample type	Adherent cells, Suspension cells
Assay type	Cell-based
Species reactivity	**Reacts with:** Mammals, Other species
Product overview	Nitric Oxide Assay Kit (Fluorometric - Orange) (ab219932) is a sensitive fluorometric assay to monitor intracellular nitric oxide (NO) levels in live cells. The assay uses an orange dye that can react with NO to generate a bright orange fluorescent product that can be easily detected at Ex/Em = 540/590 nm, using the same filter set as Cy3® or TRITC. The assay can be detected by fluorescence microscopy, microplate fluorometry or high-content imaging. It can be easily adapted to use in 384-well microplate format.
Notes	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.
Platform	Microplate reader, Fluor. microscope, Flow cyt.

Properties

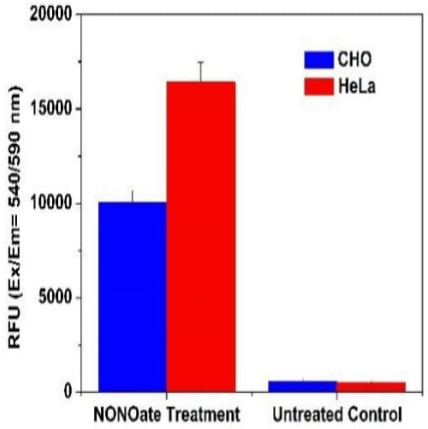
Storage instructions	Store at -20°C. Please refer to protocols.

Components	200 tests
500X NO Orange Dye	1 x 50µl
Assay Buffer I	1 x 20ml
Assay Buffer II	1 x 20ml

Relevance

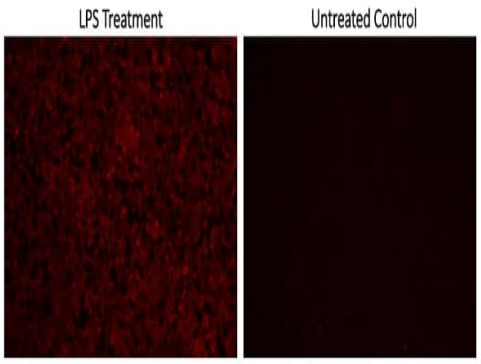
Nitric oxide (NO) is a key vertebrate biological messenger, playing an important role in neurotransmission, vascular regulation, immune responses and apoptosis. NO , also known as "endothelium-derived relaxing factor" or "EDRF", is synthesized from L-arginine, oxygen and NADPH by various NO synthases. Most of the NO in the cell is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-), and therefore the concentrations of these anions are generally as a quantitative measure of NO production.

Images



Nitric Oxide Assay Kit (Fluorometric-Orange) (ab219932)

Exogenous nitric oxide (NO) production upon DEA/NONOate treatment (NO donor). CHO-K1 (blue) and HeLa (red) cells were incubated with NO stain working solution at 37ºC for 30 minutes, and then removed to stop the staining. Cells were further treated with or without 1mM DEA/NONOate in HBSS buffer (with 10 mM HEPES (pH=6.2)) at 37ºC for 30 minutes. The solution in each well was removed and Assay Buffer II was added before measuring fluorescence. The fluorescence signal was monitored at Ex/Em = 540/590 nm (cut off = 570 nm) with bottom read mode using a FlexStation microplate reader (Molecular Devices).



Nitric Oxide Assay Kit (Fluorometric-Orange) (ab219932)

Endogenous nitric oxide (NO) production in RAW 264.7 macrophage cells. Raw 264.7 cells were seeded overnight (10^5 cells/well/100 µL) in a black wall/clear bottom 96-well plate. Left: cells were treated with 20 µg/mL of lipopolysaccharide (LPS) and 1 mM L-Arginine (L-Arg) and co-incubated with 0.5X NO stain working solution at at 37ºC for 16 hours. Right: control RAW 264.7 cells were left untreated in cell culture medium and co-incubted with 0.5X NO stain working solution at 37ºC 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 TRITC filter.

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