

Product datasheet

Nitric Oxide Assay Kit (Fluorometric) ab65327

12 References 4 Images

Overview

<b>Product name</b>	Nitric Oxide Assay Kit (Fluorometric)
<b>Detection method</b>	Fluorescent
<b>Sample type</b>	Urine, Serum, Plasma, Other biological fluids, Tissue Extracts, Cell Lysate
<b>Assay type</b>	Quantitative
<b>Assay time</b>	3h 00m
<b>Product overview</b>	Nitric Oxide Assay Kit (Fluorometric) ab65327 provides an accurate, convenient measure of total nitrate/nitrite concentration in a simple two-step process.

In the nitric oxide assay protocol:

- the first step converts nitrate to nitrite by nitrate reductase
- in the second step, nitrite reacts with the fluorescent probe DAN (2, 3 diamionaphthalene)

The fluorescence can be measured at Ex/Em = 360/450 nm. NaOH enhances the fluorescent yield. The fluorescent intensity is proportional to the total nitric oxide production. The kit has been tested with culture media, plasma, and tissue homogenates.

Nitric oxide assay protocol summary:

- add samples and standards to wells
- add enzyme cofactor, to measure nitrite only add assay buffer or to measure total nitrate + nitrite add nitrate reductase
- incubate for 1-4 hr at room temp to convert nitrate to nitrite
- add enhancer and incubate for 30 min at room temp
- add DAN probe and incubate for 10 min at room temp
- add NaOH and incubate for 10 min at room temp
- analyze with microplate reader

Notes

If you are interested in a colorimetric detection kit, please check [Nitric Oxide Assay Kit \(Colorimetric\) \(ab65328\)](#).

For an assay kit to detect nitrite alone see [Griess Reagent Kit ab234044](#).

Nitric oxide (NO) plays an important role in neurotransmission, vascular regulation, immune response and apoptosis. Since NO is rapidly converted to nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>), the total concentration of nitrite and nitrate is used as a quantitative measure of NO production.

Platform

Microplate reader

## Properties

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### Storage instructions

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

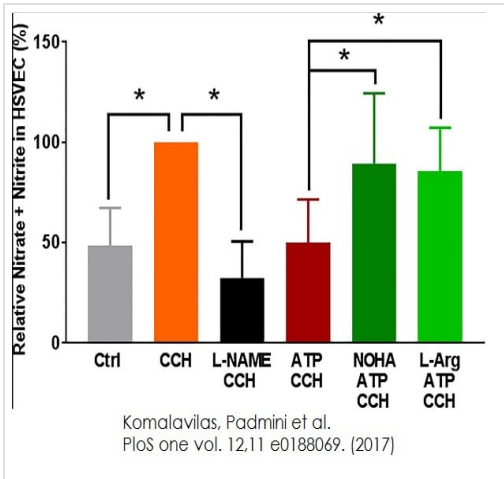
Components	Identifier	200 tests
Assay Buffer	WM	1 x 40ml
DAN Probe	Amber Red	1 x 1ml
Enhancer (Lyophilized)	Purple	1 vial
Enzyme Cofactor (Lyophilized)	Blue	1 vial
Microtiter plate		2 units
Nitrate Reductase (Lyophilized)	Green	1 vial
Nitrate Standard (Lyophilized)	Yellow	1 vial
Nitrite Standard (Lyophilized)	Orange	1 vial
Plate Cover		2 units
Sodium Hydroxide	Clear	1 x 1ml

### 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 ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ), and therefore the concentrations of these anions are generally as a quantitative measure of NO production.

### Images

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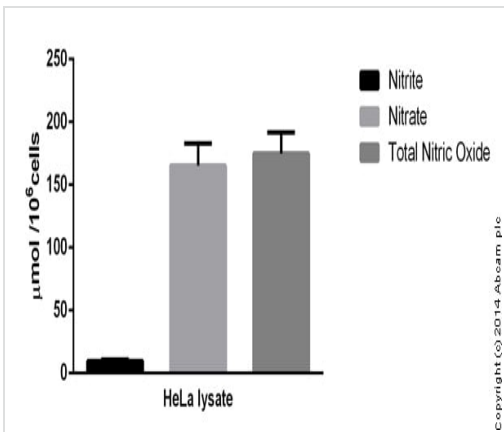


Effect of arginase inhibitor and L-arginine on eATP-induced reduction of nitric oxide production in HSV endothelial cells. HSVEC were either untreated (Ctrl), treated with ATP (2 mM), L-NAME, (100  $\mu$ M), ATP with L-arginine (L-Arg, 2 mM), or ATP with NOHA (10  $\mu$ M), for 2 hours. The cells were then stimulated with carbachol (CCH, 1  $\mu$ M) for 10 minutes and the nitric oxide generated was measured as nitrite using the NO assay kit (ab65327) and relative percent of NO generated was calculated. NO generated with CCH was set as 100%, n = 6 passages,  $\square$  p < 0.05, (One way ANOVA).

#### Functional Studies - Nitric Oxide Assay Kit

(Fluorometric) (ab65327)

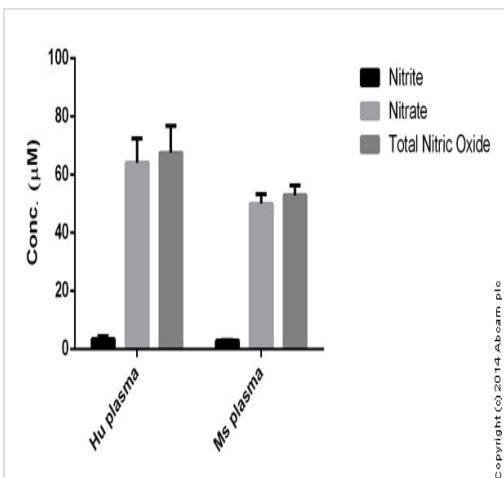
Komalavilas, Padmini et al., PloS one vol. 12,11 e0188069., Fig 7, doi:10.1371/journal.pone.0188069



Nitric oxide measured in cell lysates showing quantity (micromol) per  $10^6$  cells tested

#### Functional Studies - Nitric Oxide Fluorimetric Assay

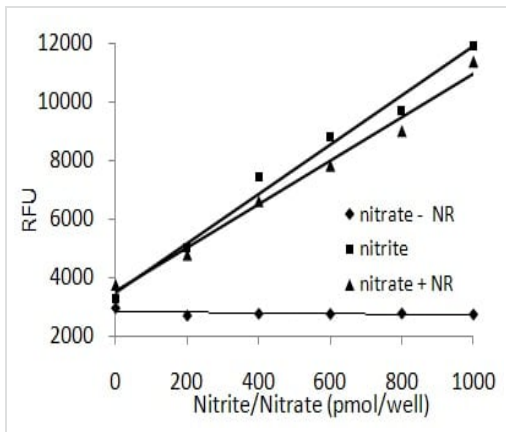
Kit (ab65327)



Nitric oxide measured in biologicals (average of 1:6 and 1:12 dilutions) showing concentration (micromolar)

#### Functional Studies - Nitric Oxide Fluorimetric

Assay Kit (ab65327)



Nitrite, nitrate assay in the presence and absence of nitrate reductase. Assays were performed according to the kit protocol with 1 hour conversion of nitrate to nitrite at Step 5.

Functional Studies - Nitric Oxide Fluorometric Assay Kit (ab65327)

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