

Nitric Oxide Assay Kit (Fluorometric) ab65327

[22 References](#) [4 Images](#)

Overview

Product name	Nitric Oxide Assay Kit (Fluorometric)
Detection method	Fluorescent
Sample type	Urine, Serum, Plasma, Other biological fluids, Tissue Extracts, Cell Lysate
Assay type	Quantitative
Assay time	3h 00m
Product overview	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 diaminonaphthalene)

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 This product is manufactured by BioVision, an Abcam company and was previously called K252 Nitric Oxide Fluorometric Assay Kit. K252-200 is the same size as the 200 test size of ab65327.

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 (NO₂⁻) and nitrate (NO₃⁻), the

total concentration of nitrite and nitrate is used as a quantitative measure of NO production.

Platform

Microplate reader

Properties

Storage instructions

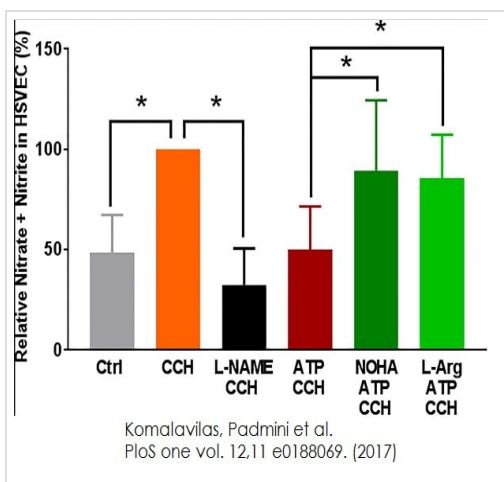
Store at -20°C. Please refer to protocols.

Components	200 tests
96-Well White Plate	2 units
DAN Probe	1 x 1ml
Enhancer II	1 vial
Microplate Sealing Film	2 units
Nitrate Reductase II	1 vial
Nitrate Standard	1 vial
Nitric Oxide Assay Buffer	1 x 40ml
Nitrite Standard	1 vial
NOS Cofactor I	1 vial
Sodium Hydroxide	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 (NO_2^-) and nitrate (NO_3^-), and therefore the concentrations of these anions are generally as a quantitative measure of NO production.

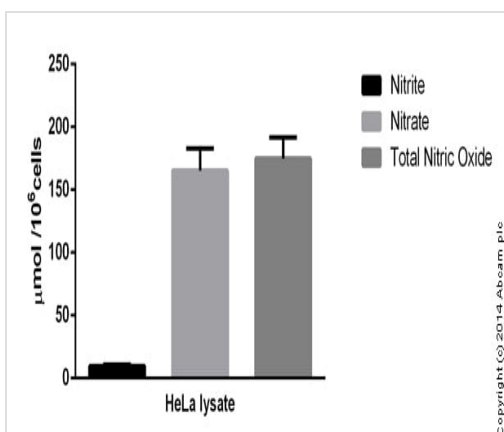
Images



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 μ M), ATP with L-arginine (L-Arg, 2 mM), or ATP with NOHA (10 μ M), for 2 hours. The cells were then stimulated with carbachol (CCH, 1 μ 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, $p < 0.05$, (One way ANOVA).

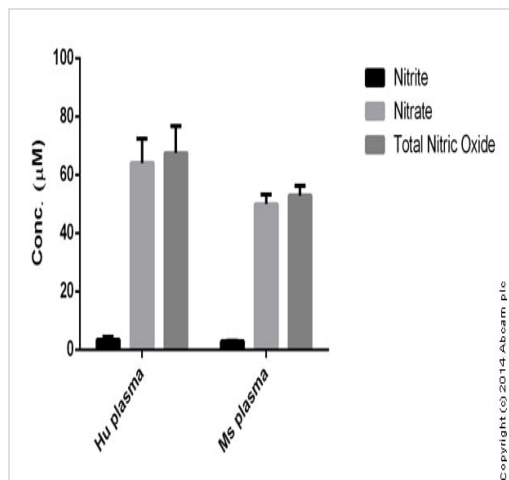
Functional Studies - Nitric Oxide Assay Kit (Fluorometric) (ab65327)

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



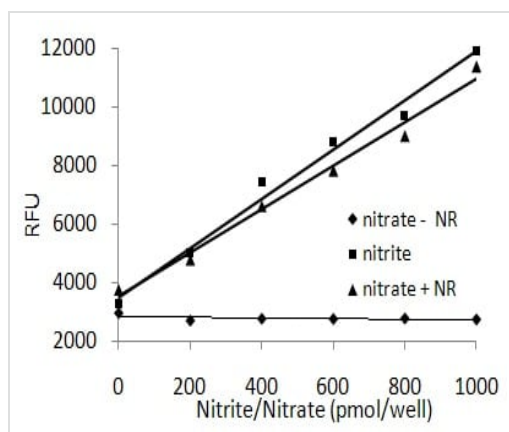
Nitric oxide measured in cell lysates showing quantity (micromol) per 10^6 cells tested

Functional Studies - Nitric Oxide Fluorimetric Assay Kit (ab65327)



Functional Studies - Nitric Oxide Fluorometric Assay Kit (ab65327)

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



Functional Studies - Nitric Oxide Fluorometric 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.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors