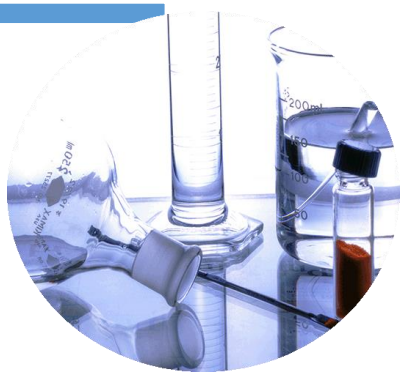
Nitric Oxide Detection Kit
INSTRUCTION MANUAL

Assays kits



Nitric Oxide Detection Kit

The Nitric Oxide Detection Kit is a ready-to-use assay kit for quantitative measurement of Nitrites in biological samples (cell culture supernatants, organic fluids...).

	Content	Catalog Number	Number of assays (96-well plate)
Nitric Oxide Detection Kit	25 mL reagent 1 (sulf.) 25 mL reagent 2 (NED) 1 mL Nitrite Standard (0.1 M)	NOS0500	**500**

For any technical questions, contact us at tech@ozbiosciences.com

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1. Technology

1.1. Description

The Nitric Oxide Detection Kit is a colorimetric assay for the determination of Nitric Oxide (NO) concentrations based on the enzymatic conversion of nitrate to nitrite by a nitrate reductase.
NO are important physiological messengers and effector molecules involved in many physiological processes such as vasodilation, inflammation, thrombosis or immunity (...). This assay is based on the reaction originally described by Griess to detect NO_2^- in a variety of biological fluids such as cell culture medium, plasma, serum, or urine.

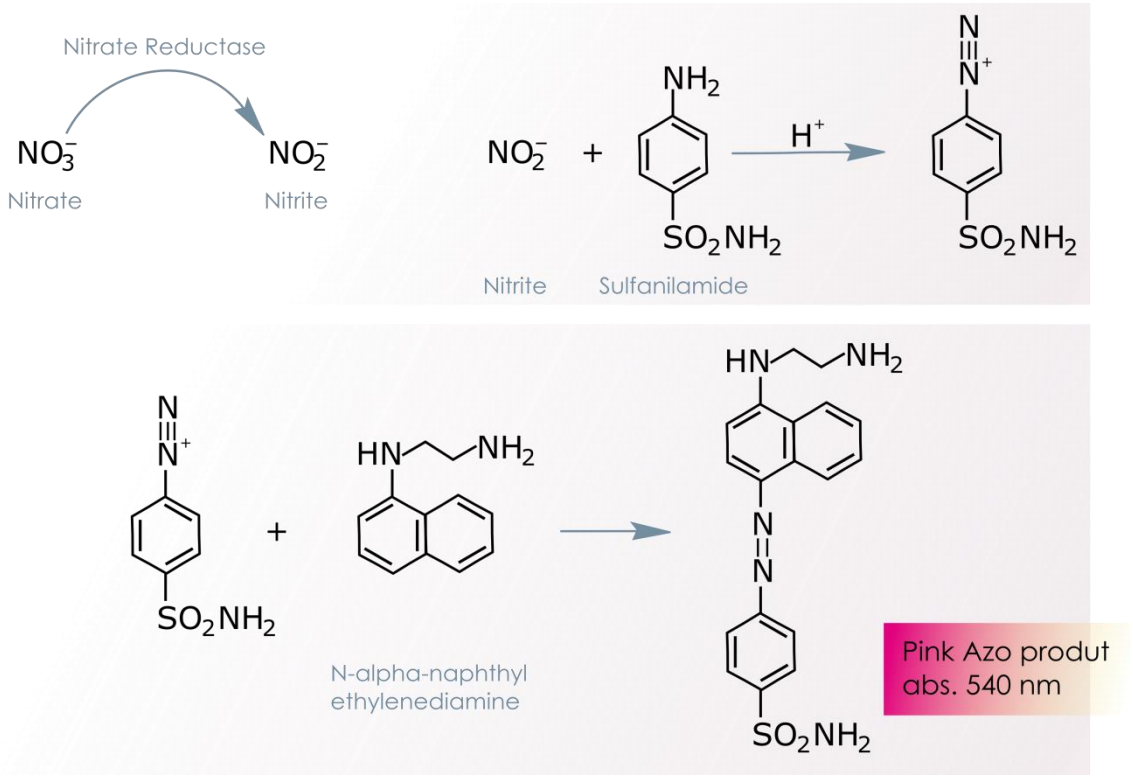


Figure 1: Nitrite quantification using the Nitric Oxide Detection Kit assay.

The Nitric Oxide Detection Kit uses sulfanilamide and N-alpha-naphthyl-ethylenediamine in phosphoric acid. Acidified NO_2^- first produces a nitrosating agent, which reacts with sulfanilic acid to produce a diazonium ion. This ion then reacts with N-alpha-naphthyl-ethylenediamine to finally yield a pink azo dye that can be measured spectrophotometrically using its absorbance at 540-570 nm.

Highly sensitive (limit of detection ~ 2µM – 125 pmol), this colorimetric kits detects NO_2^- in a linear and stable way.

1.2. Storage and shipping condition

Storage: Upon reception, store the Nitric Oxide Detection Kit at 4°C – DO NOT FREEZE. Protect from light.
Stability: 6 months.

Shipping condition: The kit is shipped at RT.

2. Applications and Protocols

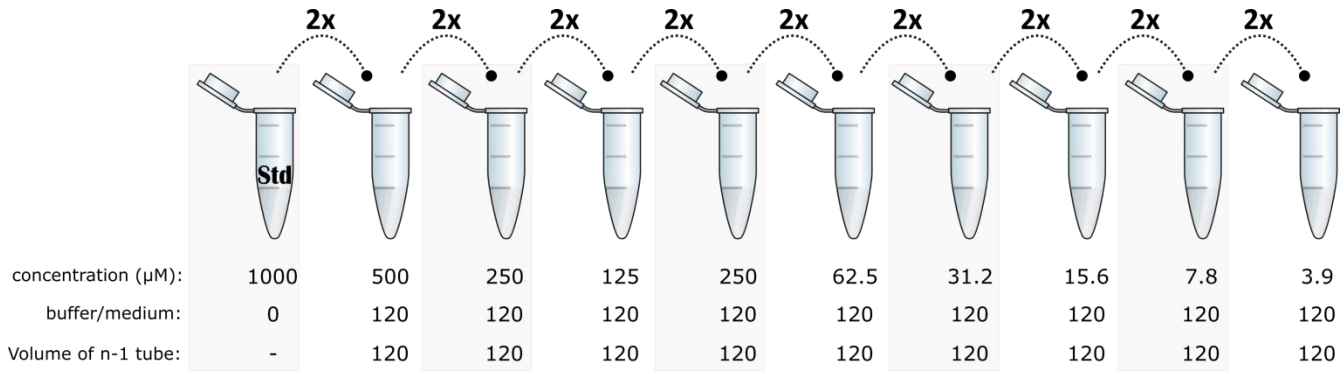
2.1. General Considerations

- This kit is not compatible with cell culture medium containing phenol red that impairs colorimetric detection: for titration of cell culture supernatants, cultivate cells in a medium without phenol red.
- Allow reagents to reach room temperature before starting.
  Avoid direct exposure to- and protect from light.
- Reagent 2 (NED) may change color if not correctly protected from light; this color change should not affect product performance significantly.
- Do not premix reagents 1 & 2 prior to the experiment: the working solution must be prepared extemporaneously.

2.2. Nitrite Standard curve preparation

Use the same buffer or medium for standard curve preparation than for experimental samples.

1. Prepare 1 mL of a 1 mM sodium nitrite oxide standard (Std) by diluting the 0.1M Nitrite standard 1:100.
2. Prepare 9 tubes containing 120 µL of buffer or medium.
3. Add 120 µL of 1 mM sodium nitrite oxide Std to the first tube and perform serial dilutions according to the protocol below.



concentration (µM):	1000	500	250	125	250	62.5	31.2	15.6	7.8	3.9
buffer/medium:	0	120	120	120	120	120	120	120	120	120
Volume of n-1 tube:	-	120	120	120	120	120	120	120	120	120

Figure 2: Recommendation for performing serial dilution using standard sodium nitrite.

4. Add 50 µL of each tube to two wells of a 96-well plate (duplicates).
5. Add 50 µL of buffer or medium in two supplementary wells for blank controls.

2.3. General protocol for nitrite measurement

1. Prepare NO Working Solution (NO-WS) by mixing equal volumes of reagent 1 (sulf.) and reagent 2 (NED); refer to table 1 below.
2. Add 50 µL of each experimental sample to wells in duplicate.

Table 1: volumes to consider for preparing NO Working Solution

Number of assays	Number of wells (duplicate)	Std curve + blank (duplicate)	Total wells (samples + std)	WS volume to prepare	Volume of reagent 1	Volume of reagent 2
1	2	22	24	1.2 mL	600 µL	600 µL
5	10	22	32	3.2 mL	1.6 mL	1.6 mL
10	20	22	42	4.2 mL	2.1 mL	2.1 mL
25	50	22	72	7.2 mL	3.6 mL	3.6 mL
35	70	22	92	9.2 mL	4.6 mL	4.6 mL

3. Dispense 50 µL of NO-WS to all experimental samples and control wells (standard curve and blank).
4. Incubate 10 min at Room Temperature (RT), protect from light.
5. Within 1 hour, measure absorbance at 540-570 nm.
6. Subtract background from all values
7. Create a standard curve by plotting average absorbance at 540-570nm over nitrite standard amount.
8. Determine NO production using the standard curve.

2.4. Optimizing sensitivity

The two reagents can be added sequentially in order to reach higher sensitivity according to the protocol below:

1. Add 50 µL of each experimental sample to wells in duplicate.
2. Dispense 25 µL of reagent 1 to all experimental samples and control wells (standard curve and blank).
3. Incubate 5-10 min at RT, protect from light.
4. Dispense 25 µL of reagent 2 to all experimental samples and control wells (standard curve and blank).
5. Incubate 5-10 min at RT, protect from light.
6. Within 1 hour, measure absorbance at 540-570 nm.
7. Subtract background from all values.
8. Create a standard curve by plotting average absorbance at 540-570nm over nitrite standard concentration.
9. Determine NO production using the standard curve.

2.5. Performance characteristics

Signal Stability.

We recommend incubating NO-WS with samples for 10 min at RT and to perform reading within 1h as signal is stable over that period of time:

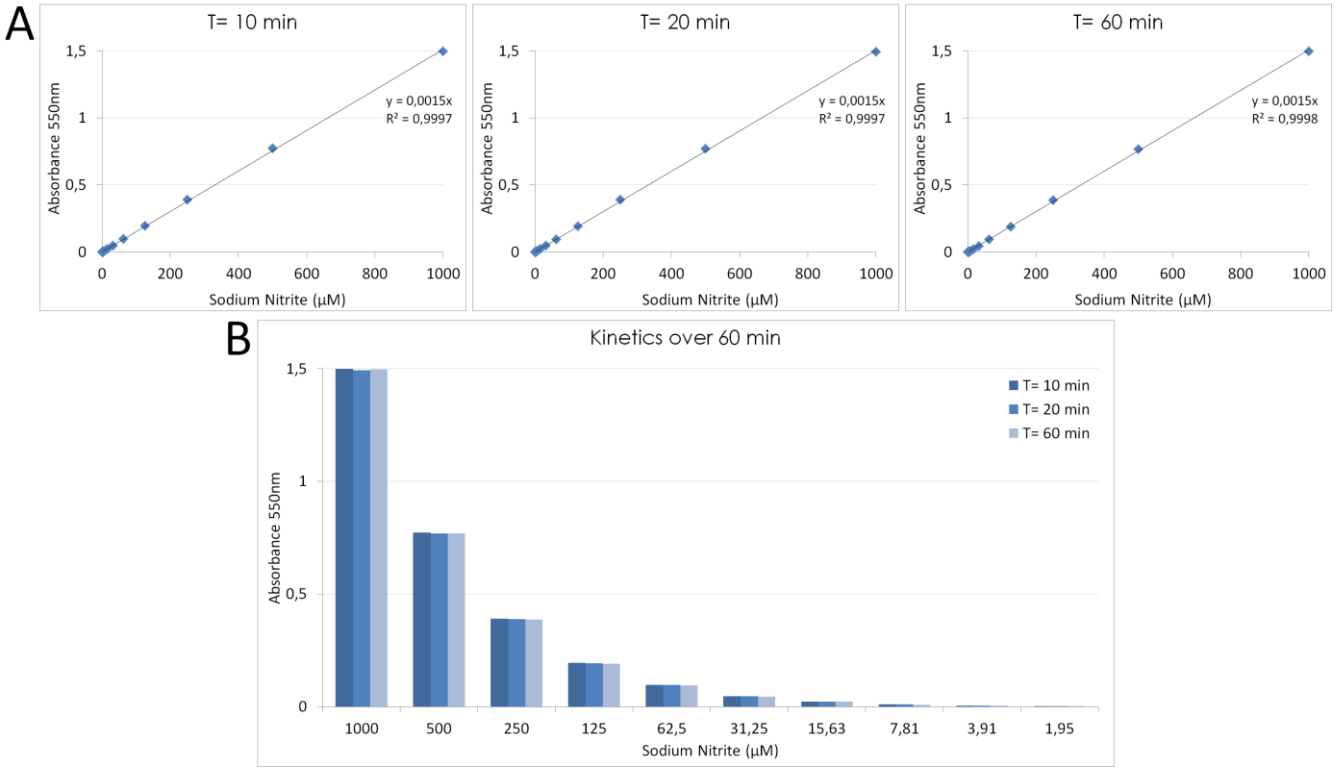


Figure 3: Nitric Oxide Detection Kit stability: sodium nitrite standard curve was prepared according to the protocol above and absorbance was read 10 to 60 min after addition of NO-WS. Over 1 hour, (A) Linearity and sensitivity are conserved and (B) no loss of signal is observed.

Recommendation for cell culture medium.

We do recommend to use only medium without phenol red to measure NO production in cell culture supernatants as phenol red interferes with colorimetric detection of NO.

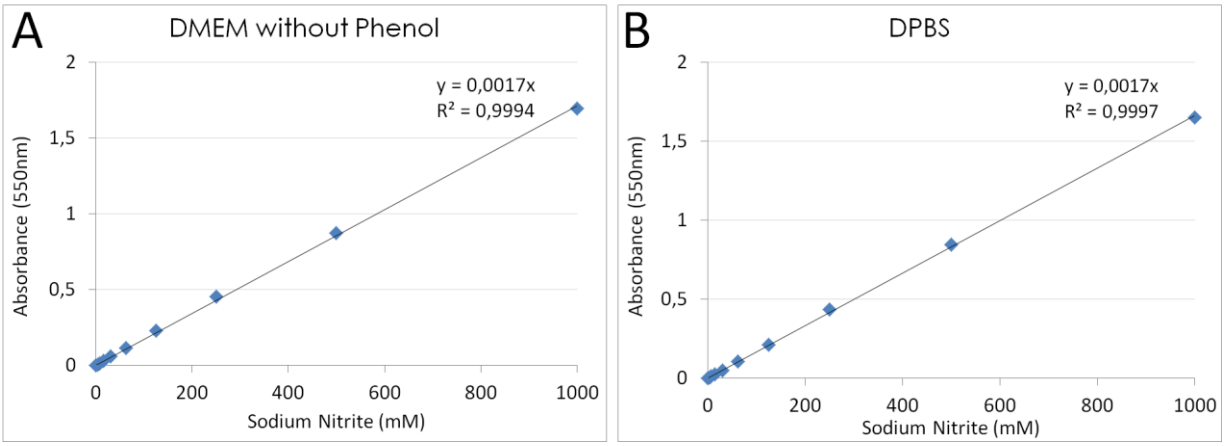


Figure 4: Nitric Oxide Detection kit is compatible with medium without phenol red. Nitrite standard curves were prepared using DMEM without phenol red (A) or DPBS (B). After addition of NO-WS and a 10 min incubation time at RT, absorbance was read at 540 nm. Results showed the exact same results between the two conditions.

NO dosage in cell supernatant.

Nitric Oxide Detection Kit was used to determine NO production in supernatants of Jurkat T cells stimulated with ranging doses of dexamethasone (from 1 to 100 µM) for 24 hours in presence or not of the nitric oxide inhibitor, L-NAME. After a centrifugation step to remove suspension cells, 50 µL of cell supernatants were tested for NO production according to the standard protocol. Absorbance was plotted over standard curve to determine quantity of NO produced. Results demonstrated a dose response in NO production in Jurkat T cells stimulated with dexamethasone (up to 50µM); this production is reduced with the addition of 50 µM L-name.

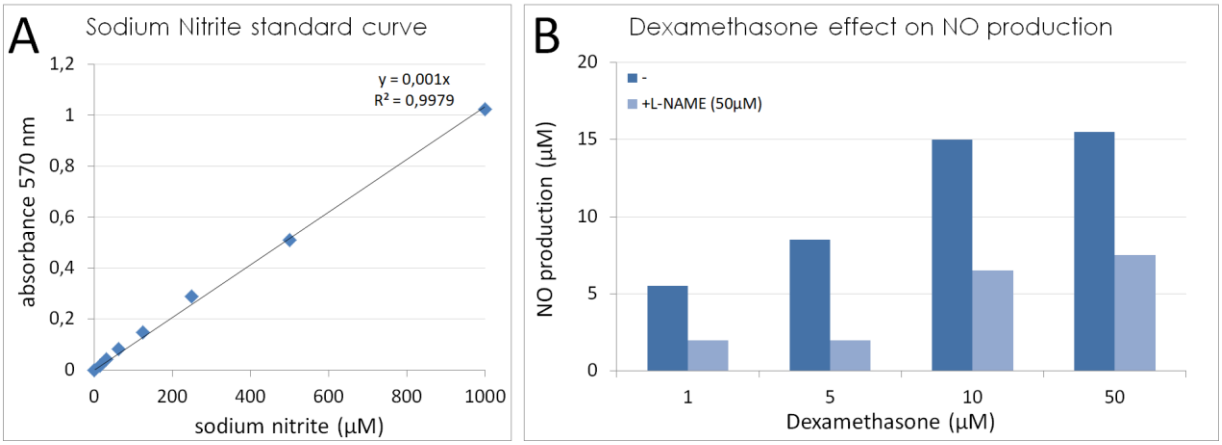


Figure 5: NO production measurement in cell culture supernatants. Nitrite standard curves generated in RPMI without phenol red (A) was used to determine level of NO produced in cell supernatants of jurkat T cells stimulated with dexamethasone in presence or not of 50 µM L-NAME (B).

3. Related Products

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Senescence Kit for Stem Cells
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