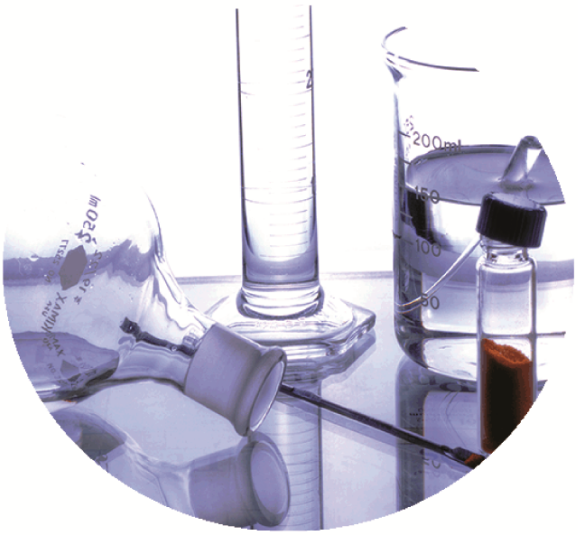

Assay kits

Nitric Oxide Detection Kit

For quantitative measurement of Nitrites in biological samples

Protocol

OZBIOSCIENCES
The art of delivery systems

IMPORTANT NOTES – Before you begin

- ✓ Each Nitric Oxide Detection Kit Assay is suitable for approximately 500 assays in 96-well plates
- ✓ This kit is not compatible with cell culture medium containing phenol red.
- ✓ Allow reagents to reach room temperature before beginning
- ✓ Avoid direct exposure to- and protect from light
- ✓ Do not premix reagents 1 & 2 prior to the experiment: the working solution must be prepared extemporaneously.
- ✓ Reagent 2 (NED) may change color if not correctly protected from light; this color change should not affect product performance significantly.

For additional information and protocols (optimization, scaling…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

tech@ozbiosciences.com

Package content	25 mL reagent 1 (sulf.), 25 mL reagent 2 (NED) 1 mL Nitrite Standard (0.1 M) Number of assays (96-well plate): 500
Shipping conditions	The kit is shipped at RT.
Storage conditions	Upon reception, store the Nitric Oxide Detection Kit at 4°C – DO NOT FREEZE. Protect from light
Shelf life	6 months from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures. During long term storage or upon shipping in cold weather.

Protocol

1. 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 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 standard to the first tube and perform serial dilutions according to the protocol below:

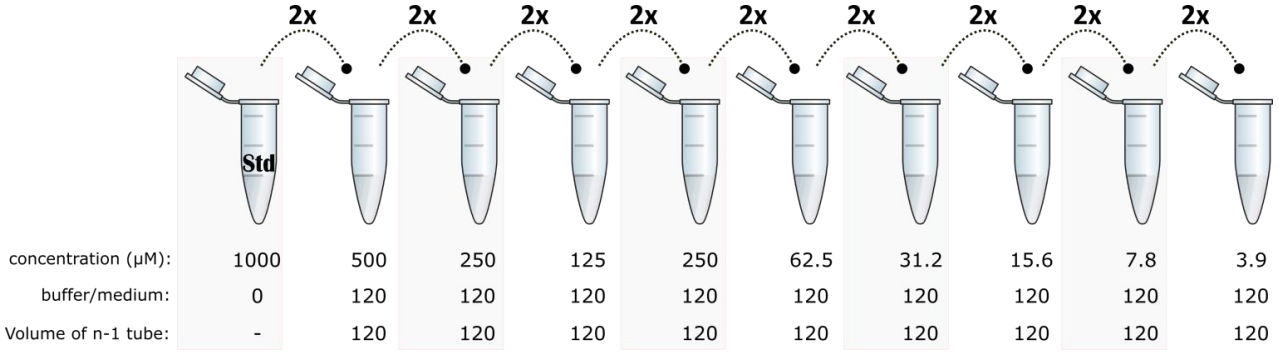

Figure 1: Recommendation for performing serial dilutions using standard sodium nitrite

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. 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.
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.

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

Table 1: volumes to consider for preparing NO Working Solution

IMPORTANT NOTE: Optimizing sensitivity

In order to **optimize 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.

NOTES

NOTES

Additional products

- **ROS Detection Assay Kit** for quantitative measurement of cellular Reactive Oxygen Species (ROS) in cells
- **MTT Cell Proliferation Assay Kit** for measuring toxicity and cell viability

Purchaser Notification

Limited License

The purchase price paid for Nitric Oxide Detection Kit Assay by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). These reagents are intended **for internal research only** by the buyer. Such use is limited to the use in the product manual. Furthermore, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Purchasers may terminate this License at any time by returning all Nitric Oxide Detection Kit Assay material and documentation to OZ Biosciences, or by destroying all Nitric Oxide Detection Kit Assay components. Purchasers are advised to contact OZ Biosciences with the notification that a Nitric Oxide Detection Kit Assay is being returned in order to obtain a refund and/or to expressly terminate a research only license granted through the purchase of the kit(s). This document covers in full the terms of the Nitric Oxide Detection Kit Assay research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

The Nitric Oxide Detection Kit Assay and all its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the kit components by following appropriate research lab practices.

EUROPE & ASIA
OZ Biosciences SAS

163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
France

Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

USA & CANADA
OZ Biosciences INC

4901 Morena Blvd
Suite 901
San Diego CA 92117
USA

Ph: + 1-858-246-7840
Fax: + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com

Rev. 06/2019