

Colorimetric Nitric Oxide Assay

Cat. No. 8098

250 tests

Introduction

Nitric Oxide (NO), produced endogenously from L-Arginine by nitric oxide synthetases, plays an important role in many physiological processes including vascular regulation, immune responses, and neural communication. NO is extremely unstable and undergoes rapid oxidative degradation to nitrite (NO₂⁻) and nitrate (NO₃⁻), which can be spectrophotometrically determined. ScienCell's Colorimetric Nitric Oxide Assay kit provides an accurate measurement of NO level in a simple two-step process: the reduction of nitrate to nitrite by vanadium (III) chloride, followed by quantification of nitrite by Griess reaction.

Kit Components

Cat. No.	# of vials	Name	Quantity	Storage
8098a	1	Nitrate Standard, 200 μM	2.5 ml	4°C, dark
8098b	1	Vanadium Chloride	25 ml	4°C, dark
8098c	1	Griess Reagent I	12.5 ml	4°C, dark
8098d	1	Griess Reagent II	12.5 ml	4°C, dark
8098e	1	20× ZnSO ₄	1.25 ml	4°C

Quality Control

The ScienCellTM Colorimetric Nitric Oxide Assay is applied to nitrate standards serially diluted from 200 to 3.13 μM. Standard curves obtained with different incubation time/temperature are shown in Figure 1.

Procedures

A. Deproteination of samples

1. Mix 285 μl of each sample (e.g. cell culture supernatant) with 15 μl of 20× ZnSO₄ in a 1.5 ml micro tube, vortex for 1 minute, centrifuge at 10,000 RCF for 10 min at 4°C, and transfer 100 μl/well of supernatant into each wells of the 96-well plate. We recommend that you prepare three replicates for each sample.

B. Preparation of nitrate standards

1. Obtain 8 test tubes and label them A through H. Add 300 μl of DI H₂O into tubes B through H.
2. Add 300 μl of the 200 μM Nitrate Standard solution into tube A.
3. Add 300 μl of the 200 μM Nitrate Standard solution into tube B and mix well to get the 100 μM nitrate standard.
4. Transfer 300 μl of the 100 μM Nitrate standard from tube B to tube C and mix well to get the 50 μM nitrate standard.
5. Repeat step 3 for tubes D-G to serially dilute the nitrate standards. Do not add any nitrate solution to tube H, which serves as the blank.
6. Obtain a 96-well test plate; prepare 3 replicates of each nitrate standard by aliquoting 100 μl/well of each nitrate standard into triplicate wells of the 96-well plate, according to the plate format shown in Table 1.

C. Measurement of $\text{NO}_3^-/\text{NO}_2^-$

1. Make fresh reaction “cocktail” by mixing 100 μl of vanadium chloride with 50 μl of Griess reagent I and 50 μl of Griess reagent II for each well of 96-well plate. Prepare adequate reaction “cocktail” based on the number of samples/standards to be assessed.
2. Add 200 μl of reaction “cocktail” to each well containing 100 μl of sample or nitrate standard and incubate for 30-120 min at room temperature, protected from light*. Solutions should turn a pale pink color.
3. Measure the absorbance on an ELISA plate reader with a test wavelength at 540 nm and a reference wavelength at 630 nm, and subtract the 630 nm background absorbance from the 540 nm measurement.

D. Calculation

1. Average the calibrated absorbance values ($\text{OD}_{540\text{nm}}$) of each sample, nitrate standard and blank wells.
2. Subtract the average $\text{OD}_{540\text{nm}}$ of blank from the average $\text{OD}_{540\text{nm}}$ of each sample and nitrate standard.
3. Generate the standard curve by plotting the calibrated $\text{OD}_{540\text{nm}}$ of the nitrate standards against the nitrate concentrations, as shown in Figure 1.
4. Determine the total concentration of nitrate and nitrite of each sample based on the standard curve.

*The time of incubation depends on the $\text{NO}_3^-/\text{NO}_2^-$ concentration of the samples. Dilute the samples if they are too concentrated. The color could get lost if the reaction goes too far due to too long incubation time or too concentrated samples. The time of incubation can be shortened by incubating at 37°C instead of room temperature.

	Nitrate standards			Samples								
	#1	#2	#3									
A	200 μ M	200 μ M	200 μ M									
B	100 μ M	100 μ M	100 μ M									
C	50 μ M	50 μ M	50 μ M									
D	25 μ M	25 μ M	25 μ M									
E	12.5 μ M	12.5 μ M	12.5 μ M									
F	6.25 μ M	6.25 μ M	6.25 μ M									
G	3.13 μ M	3.13 μ M	3.13 μ M									
H	Blank	Blank	Blank									

Figure 2. Plate format of nitrate standards and samples in NO assay.

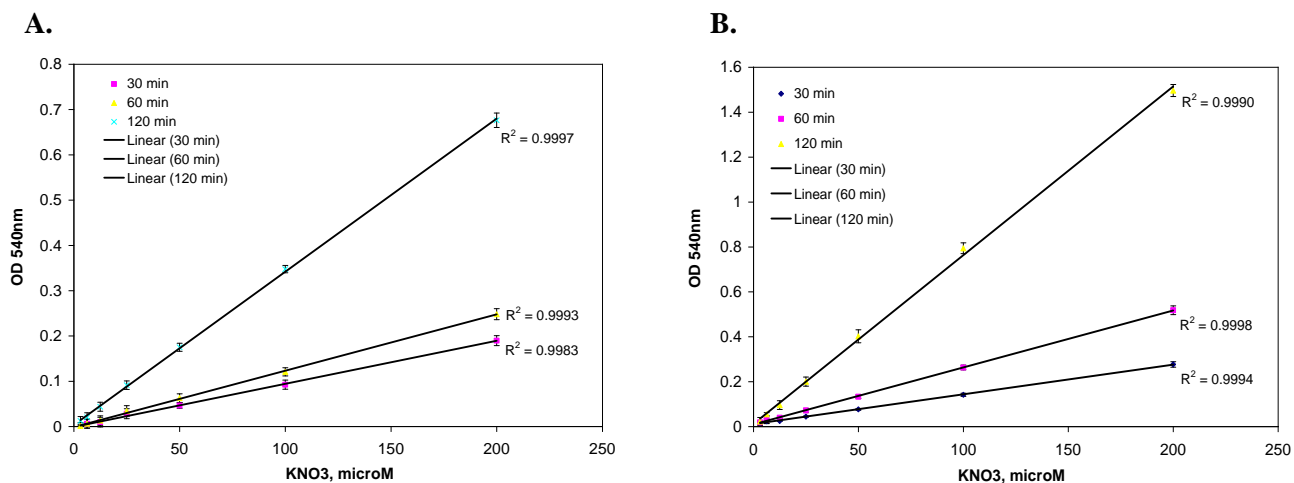


Figure 1. The ScienCell™ Colorimetric Nitric Oxide assay is applied to nitrate standards serially diluted from 200 to 3.13 μ M. Standard curves obtained with incubation time of 30, 60 and 120 min at room temperature (A) and 37°C (B) are compared.