Nitric Oxide Assay (NO)

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 repaid oxidative degradation to nitrite (NO_2^-) and nitrate (NO_3^-), which can be spectrophotometrically determined. ScienCell's 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	4ºC, dark
8098b	1	Vanadium Chloride	25 ml	4ºC, dark
8098c	1	Griess Reagent I	12.5 ml	4ºC, dark
8098d	1	Griess Reagent II	12.5 ml	4ºC, dark
8098e	1	20× $ZnSO_4$	1.25 ml	4ºC

Product Use

This assay kit is used to evaluate nitric oxide level *in vitro*. It is for research use only. Not for use in animals, humans, or diagnostic procedures.

Quality Control

The ScienCell™ 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 4ºC, 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 H_2O 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 NO_3^-/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 (OD_{540nm}) of each sample, nitrate standard and blank wells.
2. Subtract the average OD_{540nm} of blank from the average OD_{540nm} of each sample and nitrate standard.
3. Generate the standard curve by plotting the calibrated OD_{540nm} 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 NO_3^-/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 37ºC instead of room temperature.

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

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

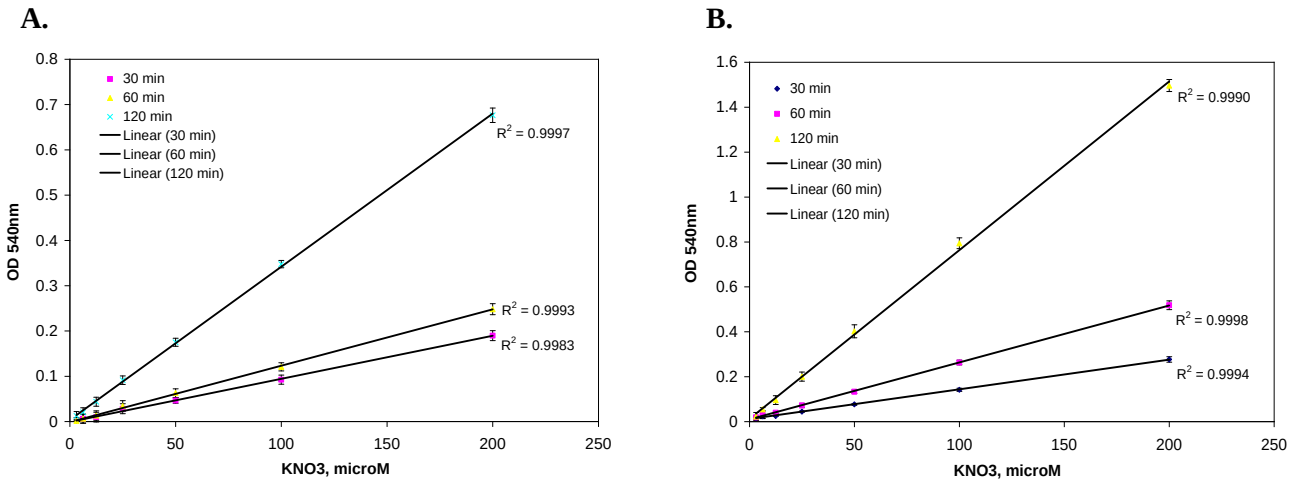

Figure 1. The ScienCell™ Nitric Oxide assay is applied to nitrate standards serially diluted from 200 to 3.13 µM. Standard curves obtained with incubation time of 30, 60 and 120 min at room temperature (A) and 37ºC (B) are compared.