ABS_Bio™ Nitric Oxide Detection Kit

(Cat# K116-100; 100 assays; store kit at -20, 4°C)

Introduction

Nitric oxide (NO) plays essential roles in mammalian life especially in defending against pathogens through oxidative toxicity. Unregulated production of nitric oxide can cause nitrosative stress, leading to damages of proteins/DNA and to cell injury and death. The generation of excessive amounts of NO can mediate protein misfolding and may cause several neurodegenerative disorders. The half-life of NO is only seconds in body fluids, and the major end products of the NO oxidation in vivo are nitrite and nitrate.

The ABS_Bio™ Nitric Oxide Detection Kit provides a simple, sensitive, colorimetric assay to detect nitrite and nitrate in a variety of samples. In this assay, $VaCl_3$ was used for nitrate-nitrite conversion, then total nitrite was measured with a modified Griess reagents. The purple color products absorption at 540 nm is directly proportional to the nitrite as an index of the NO in the sample. The kit is supplied with sufficient reagents for 100 tests in 96-well plate assay, linear detection activity range of 0.7-100 µM. It could easily be modified for use in 384-well assay and high-throughput assay.

Kit Components (100 tests)

Assay Diluent:	12 mL	Nitrite Standard:	1 mL	Reagent A :	12 mL	Griess R1:	12 mL
Griess R2:	12 mL	NaOH:	12 mL	$ZnSO_4$:	12 mL		

Storage and Handling: Store standard at -20°C, others at 4°C. Shelf Life: 6 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample preparation

Homogenize cell (2 x 10^6) or tissue (20 mg) sample in 200 µL cold assay diluent. Centrifuge to collect the supernatant. Biological liquid sample (serum, plasma, or cell lysate) need deproteinization with ultrafilter or NaOH/$ZnSO_4$. Urine can be assayed directly in 1:50 dilution with assay diluent.

Sample deproteinization: 100 µL sample, 100 µL NaOH, 100 µL ZnSO4 were added into a 1.5 mL tube, tube was vortexed and incubated at room temperature for 5 min. The tubes were then centrifuged at 14,000 rpm for 5 min at 4 °C. The collected supernatant can be measured directly by a series of dilutions of the sample with assay Diluent to ensure the readings are within the standard curve range.
It is recommended with all sample types to assay immediately or aliquot and store the samples at -80°C.
Transfer 100 µL sample into the new tubes in duplicate.

2. Standard Preparation

Transfer 100 µL Nitrite standard into 900 µL assay diluent to generate a 100 µM standard, then use the following table to generate 100, 60, 30 and 0 µM standards.

100 µM nitrite std(µL)	Assay diluent(µL)	Final nitrite concentration (µM)
300	0	100
180	120	60
90	210	30
0	300	0

Transfer 100 µL of appropriate standards into the 1.5 mL tube in duplicate. The blank control contains assay diluent only.

3. Reaction

Note: The Griess reagents react with nitrite, not nitrate.
For total nitrite/nitrate measurement: prepare enough working reagent by mixing 100 µL Reagent A, 100 µL Griess R1 and 10 µL Griss R2 for each reaction (samples and standards).
For nitrite measurement: prepare enough working reagent by mixing 100 µL assay diluent, 100 µL Griess R1 and 10 µL Griss R2 for each reaction (samples and standards).

Transfer 200 µL prepared working reagent into each reaction tube and incubate for 10 min at 60°C, or 60 min at 37°C.

4. Measurement

Quick spin the tubes and transfer 200 µL of each reaction to separate wells in a 96 well plate. Read OD540 nm.

5. Calculation

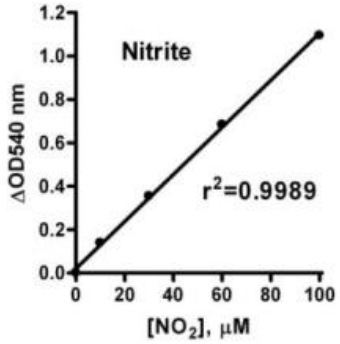
Average the duplicate OD540 nm reading for standard and sample. Subtract the average OD of the blank (OD_{blank}) from the average OD of the standards and sample, then plot the standard result (ΔOD) versus the standard concentration to get Nitrite standard curve. Determine the slope using linear regression fitting. Calculate sample Nitrite results (ΔOD_{sample}= OD_{sampe}-OD_{blank}) as the following equation.

[Total Nitrite/Nitrate] = ΔOD_{sample} /Slope x *n* (µM); [Nitrite] = ΔOD_{sample} /Slope x *n* (µM);

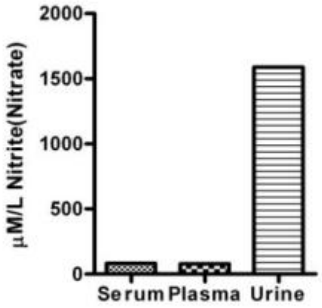
Nitrate = Total – Nitrite.

OD_{sample} and OD_{blank} are optical density values of the sample and assay diluent, respectively. *n* is the sample dilution factor (deprotein sample *n*=3).

Typical Standard Curve



Nitrite standard in 96 wells-plate assay.



Human sample in 96 wells-plate assay.

Always run your own standard curves for calculation of results.

Sensitivity and Limit of Detection

The Limit of Detection was determined as 0.7 µM, and linear detection range up to 100 µM in 96-well plate colorimetric assay. Samples with values above linear range should be diluted with assay diluent, re-assayed, and multiplied by dilution factor.

Interferences

Antioxidants (e.g. NaN_3, ascorbic acid, β-mercaptoethanol and DTT) may interfere with color development. Avoid using these compounds during sample preparation. Culture media may contains nitrate, when assay culture supernatant sample, the nitrite standard preparation should use culture media to replace assay diluent.

References

Yucel, AA. et al. 2012, J Experimental & Integrative Medicine. 2:167-171
Sun, J. et al. 2003, Sensors, 3, 276-284

Related Products:

ALP Colorimetric Detection Kit (#K109-200)
ALP Fluorimetric Detection Kit (#K110-200)
NAD/NADH Detection Kit (#K120-100)
Creatinine Detection Kit (#K148-200)
Glucose Detection Kit (#K188-100)
Glutathione Detection Kit (#K140-100)
Glutathione Peroxidase Detection Kit (#K143-100)
Glutathione Reductase Detection Kit (#K146-100)
Pyruvate Detection Kit(#K150-100)
Pyruvate Kinase Activity Detection Kit (#K151-100)
G6P Colorimetric Detection Kit (#K321-100)
Heme Detection Kit (#K169-200)
Lactate Detection Kit (#K207-100)
NADP/NADPH Detection Kit (#K123-100)
α-Glucosidase Activity Detection Kit (#K218-100)
Phosphatase Detection Kit (#K196-500)
Urea Detection Kit (#K158-200)
ADP Detection Kit (#K134-100)
ATP Detection Kit (#K133-100)
Phospho(enol)pyruvate (PEP) Detection Kit (#K309-100)
Alcohol Dehydrogenase Activity Detection Kit (#K113-100)
G6P Fluorimetric Detection Kit (#K322-100)