BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Nitric Oxide Assay Kit (Cat #: A-103)

COMPONENTS: NO Assay Solution- 50 ml, store at 4ºC (for 500 wells)
Sodium Nitrite- 1 g, store at room temperature

PRODUCT DESCRIPTION: Nitric oxide (NO) measurement can be conveniently achieved using the standard technique for measuring inorganic nitrite. This assay is based on the observation that the adduct of nitroxides and sulfanilic acid interacts with N-(1-naphthyl)ethylenediamine, generating a product that can be quantified by spectrophotometry (Anal. Biochem. 126:131,1982). Nitrite concentrations can be obtained by comparison to a standard curve performed at the same time. This simple assay (Griess assay) can be run on a microplate. The assay kit is stable for one year if stored refrigerated and shielded from light.

PROTOCOLS

Nitrite standard preparation -

Prepare a fresh 10 mM sodium nitrite solution (13.8 mg dissolved in 20 ml ddH_2O) for each assay. First dilute the 10 mM solution 1000-fold to obtain a 10 μM solution, e.g., 1 μl 10 mM nitrite + 999 μl dH_2O. Then make 1:1 serial dilution to obtain 5, 2.5, and 1.25 μM nitrite standards. Discard the standards after each assay.

Sample preparation

Cell sample: Pellet at least ~10^6 normal saline-washed cells in a microtube. Remove saline completely. Add 0.1 ml ice-cold 10% trichloroacetic acid (TCA) solution (not included) to cell pellet. Disrupt the cell pellet by vigorous trituration and vortexing. Mechanical homogenization can facilitate this step. Agitate extract on ice for 5 min. Clarify sample by centrifugation at ~13,000 rpm for 5 min. Collect supernatant for the assay.

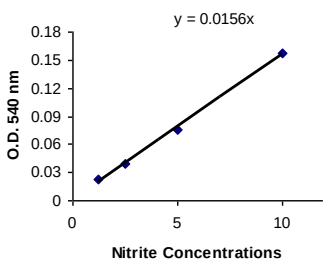
Tissue sample: Tissue sample should be washed with normal saline to remove blood residues. Tissue (~50 mg) is mechanically homogenized in 0.2 ml of ice-cold 10% TCA. Agitate extract on ice for 5 min. Clarify sample by centrifugation at ~13,000 rpm for 5 min. Collect supernatant for the assay.

Serum/plasma sample: Serum/plasma should be deproteinized by TCA precipitation. Mix sample with an equal volume of ice-cold 10% TCA and incubate on ice for 5 min. Clarify sample by centrifugation at ~13,000 rpm for 5 min. Collect supernatant for the assay.

Cell culture medium can be used directly for the assay without pretreatment.

ASSAY

1. Add 0.1 ml of dH_2O, control medium, or a suitable solution as blank to a plain (uncoated) 96-well plate.
2. Add 0.1 ml of each nitrite standard to a set of wells and samples to another set of wells.
3. Assay is initiated by adding 0.1 ml NO Assay Solution to each well. Mix contents by gentle agitation. Incubate at room temperature shielded from light for 15 min.
4. Measure absorbance (O.D.) at 540-550 nm using a plate reader. Plot nitrite standard concentrations vs. O.D.. Generate a trend line equation on chart (see graph below).
5. Calculate sample nitrite concentration using the derived equation (x = nitrite concentration in μM; y = O.D.). A new plot must be generated for each assay.



NOTE:

NO Assay Solution contains sulfanilic acid and N-(1-naphthyl)ethylenediamine. Please contact us or visit the product webpage for SDS information.