Datasheet for ABIN1000244

Nitric Oxide Assay Kit

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Overview

Quantity:	100 tests
Target:	Nitric Oxide (NO)
Application:	Biochemical Assay (BCA)

Product Details

Sample Type:	Cell Lysate, Food, Plasma, Serum, Tissue Lysate, Urine
Specificity:	0.6 µM
Characteristics:	Sensitive and accurate. Detection range 0.6 - 200 µM in 96-well plate. Rapid and reliable. Using an optimized VCl3 reagent, the time required for reduction of NO3 - toNO2 - is 10 min at 60°C. Simple and high-throughput. The procedure involves mixing sample with three reagents, incubation for 10 min at 60°C and reading the optical density. Can be readily automated to measure thousands of samples per day.
Components:	Reagent A: 12 mL. Reagent B: 500 µL. Reagent C: 12 mL. NaOH: 1 mL. ZnSO4: 1 mL. Standard: 1 mL.
Material not included:	Pipetting devices, eppendorf tubes, eppendorf centrifuge, clear, flat bottomed 96 well plates or cuvettes, plate reader or spectrophotometer and heat block or hot water bath (optional).

Target Details

Target:	Nitric Oxide (NO)
Alternative Name:	Nitric Oxide (NO Products)
Target Type:	Anorganic

Target Details

Background:	Quantitative determination of nitric oxide by colorimetric (540nm) method. Procedure: 40 min. Nitric oxide (NO) is a reactive radical that plays an important role in many key physiological functions. NO, an oxidation product of arginine by nitric oxide synthase, is involved in host defense and development, activation of regulatory proteins and direct covalent interaction with functional biomolecules. Simple, direct and automation-ready procedures for measuring NO are becoming popular in Research and Drug Discovery. Since NO is oxidized to nitrite and nitrate, it is common practice to quantitate total NO2- /NO3- as a measure for NO level. This Nitric Oxide Assay Kit is designed to accurately measure NO production following reduction of nitrate to nitrite using improved Griess method. The procedure is simple and the time required for sample pretreatment and assay is reduced to as short as 30 min.

Application Details

Application Notes:	Direct Assays: NO in plasma, serum, urine, tissue/cells and foods. Drug Discovery/Pharmacology: effects of drugs on NO metabolism.
Comment:	Antioxidants and nucleophiles (e.g. beta-mercaptoethanol, glutathione, dithiothreitol and cysteine) may interfere with this assay. Avoid using these compounds during sample preparation.
Protocol:	Sample treatment: tissue or cell samples are homogenized in 1 x PBS (pH 7.4). Centrifuge at 10,000g or higher at 4°C. Use supernatant for NO assay. Samples that need deproteination include serum, plasma, whole blood, cell culture media containing FBS, tissue or cell lysates. Urine and saliva do not need deproteination. Deproteination. Mix 150 µL sample with 8 µL ZnSO4 in1.5-mL tubes. Vortex and then add 8 µL NaOH, votex again and centrifuge 10 min at 14,000 rpm. Transfer 100 µL of the clear supernatant to a clean tube. Note: If samples need to be deproteinated, 150 µL of each standard should be prepared and also treated with ZnSO4 and NaOH to eliminate the need for a dilution factor. Procedure using 96-well plate: 1. Standards. Prepare 500 µL 100 µM Premix by mixing 50 µL 1.0 mM Standard and 450 µL distilled water. 2. Reaction. Add 100 µL of each sample to separate, labeled eppendorf tubes. (We recommend that samples be measured in at least duplicate). Immediately prior to starting the reaction, prepare enough Working Reagent (WR) for all samples and standards by mixing per reaction tube: 100 µL Reagent A, 4 µL Reagent B and 100 µL Reagent C. Add 200 µL of the WR to each

Application Details

	sample and standard tube and incubate for 10 min at 60°C. (Alternatively, the reaction can be run at 37°C for 60 min or RT for 150 min.) 3. Measurement. Briefly centrifuge the reaction tubes to pellet any condensation and transfer 250 µL of each reaction to separate wells in a 96 well plate. Read OD at 500-570nm (peak 540 nm). Procedure using Cuvette: Prepare standards and samples as described for the 96-well procedure except quadruple (4x) the volumes. After the reaction, transfer 1 mL to a cuvette. Measure OD540nm in the cuvette.
Calculation of Results:	Subtract blank OD (Std 4) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. Conversions: 1 mg/dL NO equals 333 µM, 0.001% or 10 ppm.
Restrictions:	For Research Use only

Handling

Storage:	4 °C

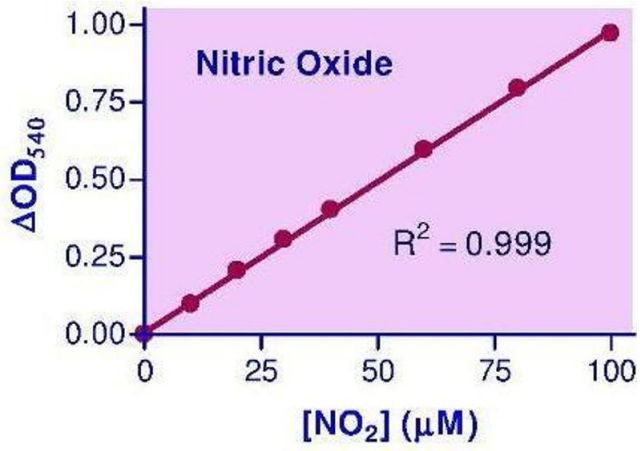
Publications

Product cited in:	Zeng, Peng, Monie, Yang, Pang, Hung, Wu: "Control of cervicovaginal HPV-16 E7-expressing tumors by the combination of therapeutic HPV vaccination and vascular disrupting agents." in: **Human gene therapy**, Vol. 22, Issue 7, pp. 809-19, (2011) (PubMed). Farfara, Trudler, Segev-Amzaleg, Galron, Stein, Frenkel: "γ-Secretase component presenilin is important for microglia β-amyloid clearance." in: **Annals of neurology**, Vol. 69, Issue 1, pp. 170-80, (2011) (PubMed). Habib, Eisa, Arafat, Marie: "Pulmonary involvement in early rheumatoid arthritis patients." in: **Clinical rheumatology**, Vol. 30, Issue 2, pp. 217-21, (2011) (PubMed). Smith, Smith, Bowlin, White: "Modulation of murine innate and acquired immune responses following in vitro exposure to electrospun blends of collagen and polydioxanone." in: **Journal of biomedical materials research. Part A**, Vol. 93, Issue 2, pp. 793-806, (2010) (PubMed). Rahman, Bhattacharya, Banu, Kang, Fernandes: "Endogenous n-3 fatty acids protect

ovariectomy induced bone loss by attenuating osteoclastogenesis." in: **Journal of cellular and molecular medicine**, Vol. 13, Issue 8B, pp. 1833-44, (2010) (PubMed).

There are more publications referencing this product on: Product page

Images



Biochemical Assay

Image 1.