

## QuantiChrom™ Nitric Oxide Assay Kit (D2NO-100)

### Quantitative Colorimetric Determination of Nitric Oxide at 540 nm

#### DESCRIPTION

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  $\text{NO}_2^-/\text{NO}_3^-$  as a measure for NO level. BioAssay Systems' QuantiChrom™ 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.

#### KEY FEATURES

**Sensitive and accurate.** Detection range 0.6 - 200  $\mu\text{M}$  in 96-well plate.

**Rapid and reliable.** Using an optimized  $\text{VCl}_3$  reagent, the time required for reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  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.

#### APPLICATIONS

**Direct Assays:** NO in plasma, serum, urine, tissue/cells and foods.

**Drug Discovery/Pharmacology:** effects of drugs on NO metabolism.

#### KIT CONTENTS (100 tests in 96-well plates)

**Reagent A:** 12 mL    **Reagent B:** 500  $\mu\text{L}$     **Reagent C:** 12 mL  
**NaOH:** 1 mL    **ZnSO<sub>4</sub>:** 1 mL    **Standard:** 1 mL

**Storage conditions.** The kit is shipped at room temperature. Store the Standard at -20°C and all other reagents at -20 to 4°C. Shelf life of six months after receipt.

**Precautions:** reagents are for research use only. Please refer to Material Safety Data Sheet for detailed information.

#### PROCEDURES

Prior to assay, equilibrate all components to room temperature. If precipitates are present in Reagent B, warm at 37°C until redissolved (~10-15 min).

**Sample treatment:** tissue or cell samples are homogenized in 1 × PBS (pH 7.4). Centrifuge at 14,000 rpm 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  $\mu\text{L}$  sample with 8  $\mu\text{L}$   $\text{ZnSO}_4$  in 1.5-mL tubes. Vortex and then add 8  $\mu\text{L}$  NaOH, vortex again and centrifuge 10 min at 14,000 rpm. Transfer 100  $\mu\text{L}$  of the clear supernatant to a clean tube. *Note: If samples need to be deproteinated, 150  $\mu\text{L}$  of each standard should be prepared and also treated with  $\text{ZnSO}_4$  and NaOH to eliminate the need for a dilution factor.*

#### Procedure using 96-well plate:

1. **Standards.** Prepare 500  $\mu\text{L}$  100  $\mu\text{M}$  Premix by mixing 50  $\mu\text{L}$  1.0 mM Standard and 450  $\mu\text{L}$  distilled water. Dilute standards in 1.5 mL centrifuge tubes as described in the Table.

No	Premix + H <sub>2</sub> O	Nitrite ( $\mu\text{M}$ )
1	250 $\mu\text{L}$ + 0 $\mu\text{L}$	100
2	150 $\mu\text{L}$ + 100 $\mu\text{L}$	60
3	75 $\mu\text{L}$ + 175 $\mu\text{L}$	30
4	0 $\mu\text{L}$ + 250 $\mu\text{L}$	0

2. **Reaction.** Add 100  $\mu\text{L}$  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  $\mu\text{L}$  Reagent A, 4  $\mu\text{L}$  Reagent B and 100  $\mu\text{L}$  Reagent C. Add 200  $\mu\text{L}$  of the WR to each 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.) *Note:* If precipitates are present in Reagent B, warm at 37°C until redissolved (~10-15 min).

3. **Measurement.** Briefly centrifuge the reaction tubes to pellet any condensation and transfer 250  $\mu\text{L}$  of each reaction to separate wells in a 96 well plate. Read OD at 500-570 nm (peak 540 nm).

#### Procedure using Cuvette:

Prepare standards and samples as described for the 96-well procedure except quadruple (4×) the volumes. After the reaction, transfer 1 mL to a cuvette. Measure OD<sub>540nm</sub> in the cuvette.

#### CALCULATION

Subtract blank OD (Std 4) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The NO concentration of Sample is calculated as

$$[\text{Nitric Oxide}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope}} \quad (\mu\text{M})$$

OD<sub>SAMPLE</sub> and OD<sub>BLANK</sub> are optical density values of the sample and water, respectively.

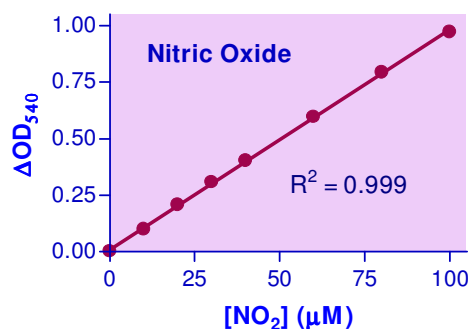
**Conversions:** 1 mg/dL NO equals 333  $\mu\text{M}$ , 0.001% or 10 ppm.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

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).

#### GENERAL CONSIDERATIONS

Antioxidants and nucleophiles (e.g.  $\beta$ -mercaptoethanol, glutathione, dithiothreitol and cysteine) may interfere with this assay. Avoid using these compounds during sample preparation.



Standard Curve in 96-well plate assay

#### LITERATURE

- Bolander Jr, F. F. (2005). The compartmentalization of prolactin signaling in the mouse mammary gland. *Mol. Cell. Endocrinol* 245:105–110.
- Bulau, P. et al. (2007). Analysis of methylarginine metabolism in the cardiovascular system identifies the lung as a major source of ADMA. *Am J Physiol Lung Cell Mol Physiol* 292: L18-L24.
- Hasegawa, K. et al (2007). Role of asymmetric dimethylarginine in vascular injury in transgenic mice overexpressing dimethylarginine dimethylaminohydrolase. *Circ Res.* 101(2):e2-10.