



# **Nitric Oxide Microplate Assay Kit**

## **User Manual**

**Catalog # CAK1063**

(Version 1.3E)

Detection and Quantification of Nitric Oxide (NO) Content in Urine,  
Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and  
Other biological fluids Samples.

**For research use only. Not for diagnostic or therapeutic procedures.**

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## I. INTRODUCTION

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 NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> as a measure for NO level.

The reaction products can be measured at a colorimetric readout at 550 nm.

## II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 1	4 °C
Assay Buffer II	30 ml x 1	4 °C
Dye Reagent A	Powder x 1	4 °C, keep in dark
Dye Reagent A Diluent	5 ml x 1	4 °C
Dye Reagent B	Powder x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

**Note:**

**Standard:** add 1 ml distilled water, mix; then add 2  $\mu$ l into 998  $\mu$ l distilled water; the concentration of the standard will be 200  $\mu$ mol/L. Store at 4°C.

**Dye Reagent A:** add 5 ml Dye Reagent A Diluent before use, mix. If any undissolved substance, please use water bath heating to dissolve it.

**Dye Reagent B:** add 5 ml distilled water before use.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

#### IV. SAMPLE PREPARATION

##### 1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5 ml distilled water for  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 12000g 4 °C for 20 minutes. Add 0.25 ml Assay Buffer I, mix; then add 0.25 ml Assay Buffer II, mix; centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

##### 2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 0.5 ml distilled water, transfer all samples into centrifuge tube, add 0.25 ml Assay Buffer I, mix; then add 0.25 ml Assay Buffer II, mix; centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

##### 3. For serum or plasma samples

Add 0.5 ml samples into centrifuge tube, add 0.25 ml Assay Buffer I, mix; then add 0.25 ml Assay Buffer II, mix; centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

## V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	50 $\mu$ l	--	--
Standard	--	50 $\mu$ l	--
Distilled water	--	--	50 $\mu$ l
Dye Reagent A	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l
Mix, incubate for 10 minutes.			
Dye Reagent B	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l
Mix, incubate for 5 minutes, measured at 550 nm and record the absorbance.			

### Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- 3) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

1. According to the weight of sample

$$\begin{aligned} \text{NO } (\mu\text{mol/g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times \\ & \quad V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 0.2 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

2. According to the quantity of cells or bacteria

$$\begin{aligned} \text{NO } (\mu\text{mol}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ & \quad V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 0.2 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

3. According to the volume of serum or plasma

$$\begin{aligned} \text{NO } (\mu\text{mol/ml}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \\ & \quad \times n \\ &= 0.4 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

$C_{\text{Protein}}$ : the protein concentration, mg/ml;

$C_{\text{Standard}}$ : the standard concentration, 200  $\mu\text{mol/L}$  = 0.2  $\mu\text{mol/ml}$

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

$V_{\text{Sample}}$ : the volume of sample, 0.05 ml;

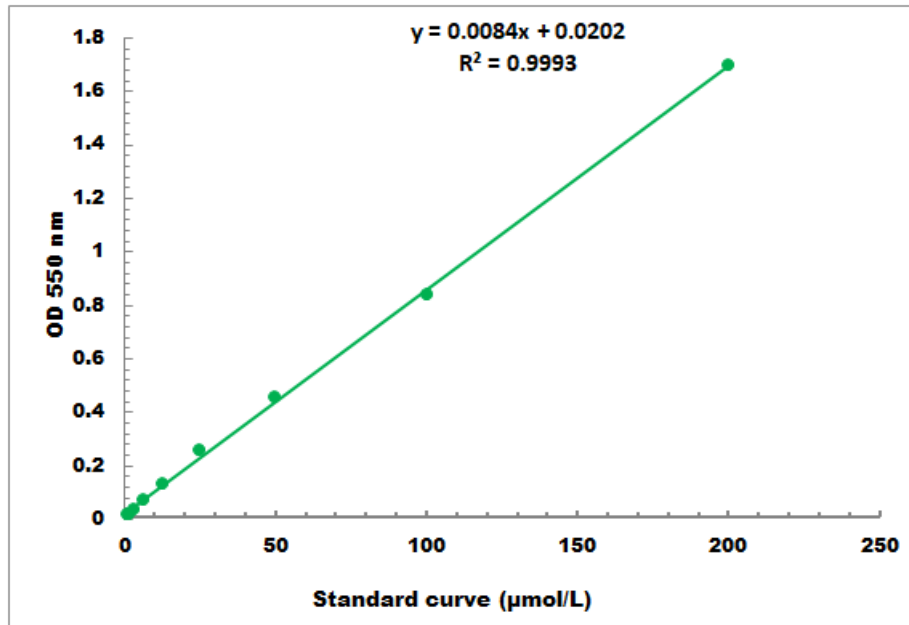
$V_{\text{Standard}}$ : the volume of standard, 0.05 ml;

$V_{\text{Assay}}$ : the volume of total buffer in sample preparation, 1 ml.

n: dilution factor, n=2.

## VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 2 µmol/L - 200 µmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES