Nitric Oxide Microplate Assay Kit

User Manual

Catalog # ASK1063

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 NO2-/ NO3- as a measure for NO level.

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

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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 µl into 998 µl distilled water; the concentration of the standard will be 200 µmol/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

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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 × 106 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.

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V. ASSAY PROCEDURE

Add following reagents into the microplate:

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

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VI. CALCULATION

1. According to the weight of sample

$$NO\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the quantity of cells or bacteria

$$NO\ (\mu mol/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

3. According to the volume of serum or plasma

$$NO\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} \times n$$

$$= 0.4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the standard concentration, 200 μmol/L = 0.2 μmol/ml

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

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

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

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

n: dilution factor, n=2.

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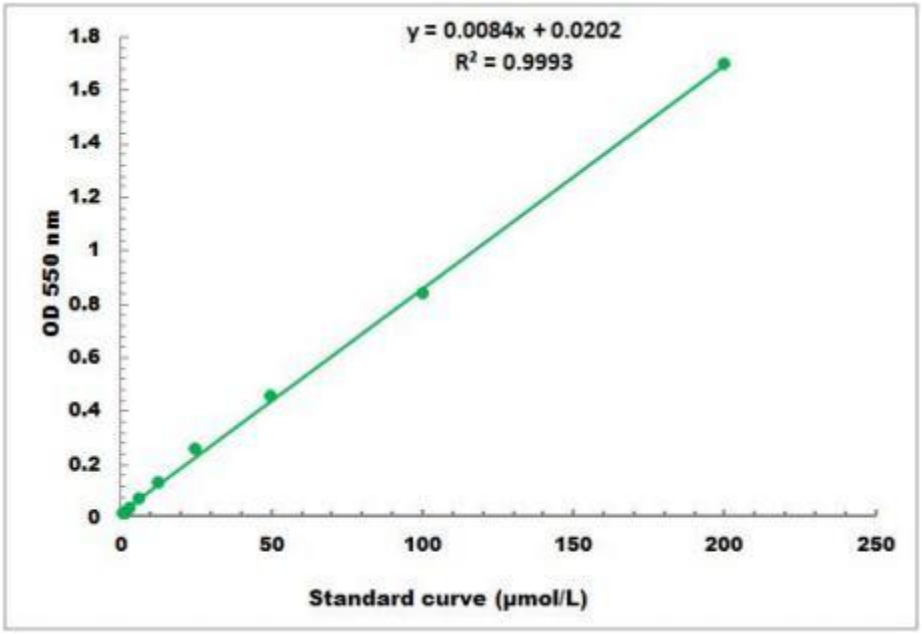
VII. TYPICAL DATA

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



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

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