



Nitric Oxide Microplate Assay Kit

Catalog # AS0063

Detection and Quantification of Nitric Oxide Content in Urine,
Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and
Other biological fluids Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic 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.

II. KIT COMPONENTS

| Component | Volume | Storage |
|-----------------------|------------|--------------------|
| 96-Well Microplate | 1 plate | |
| Assay Buffer | 30ml x 4 | 4 °C |
| Dye Reagent A | Powder x 1 | 4 °C, keep in dark |
| Dye Reagent ADiluent | 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 μ l into 998 μ l distilled water; the concentration of the standard will be 200 μ mol/L. Store at 4°C.

Dye Reagent A: add 5 ml Dye Reagent ADiluent 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
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 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 12000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 12000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

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

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{NO } (\mu\text{mol/mg}) = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(C_{\text{Protein}} \times V_{\text{Sample}}) \times (OD_{\text{Standard}} - OD_{\text{Blank}})}$$

$$= 0.2 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}}$$

2. According to the weight of sample

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

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

3. According to the quantity of cells or bacteria

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

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

4. According to the volume of serum or plasma

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

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

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

C_{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, $N \times 10^4$;

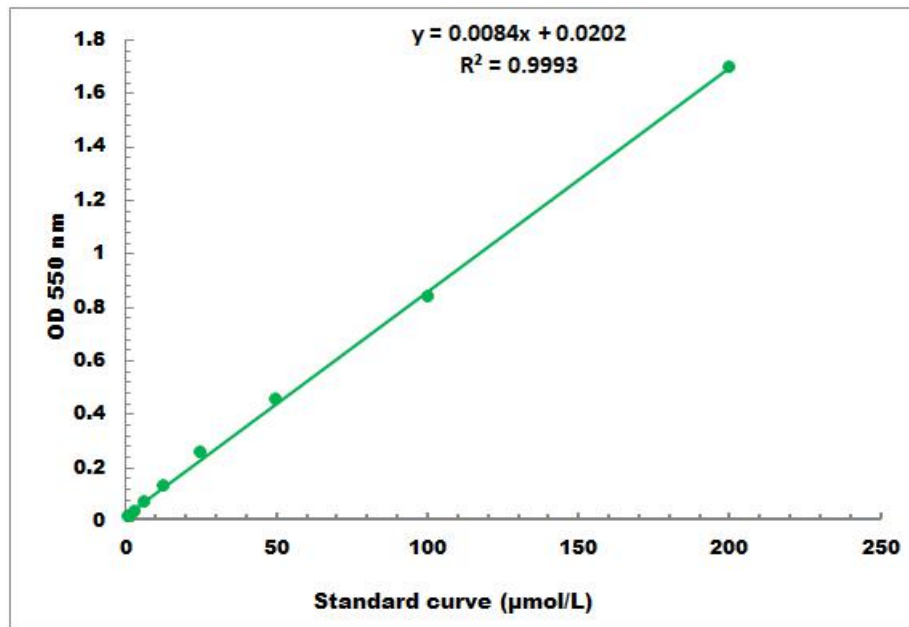
V_{Sample} : the volume of sample, 0.05 ml;

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

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

VII. TYPICAL DATA

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



Detection Range: 2 µmol/L -200µmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES