# Nitric Oxide Microplate Assay Kit

# User Manual

**Catalog # FTA0062**

(Version 1.2D)

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.**

## 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 | 30 ml x 4 | 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
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 $5 \times 10^6$ cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 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 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. | | | |

**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.

## VI. CALCULATION

1. According to the protein concentration of sample

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

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

2. According to the weight of sample

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

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

3. According to the quantity of cells or bacteria

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

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

4. According to the volume of serum or plasma

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

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

$C_{Protein}$: 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 × $10^4$;

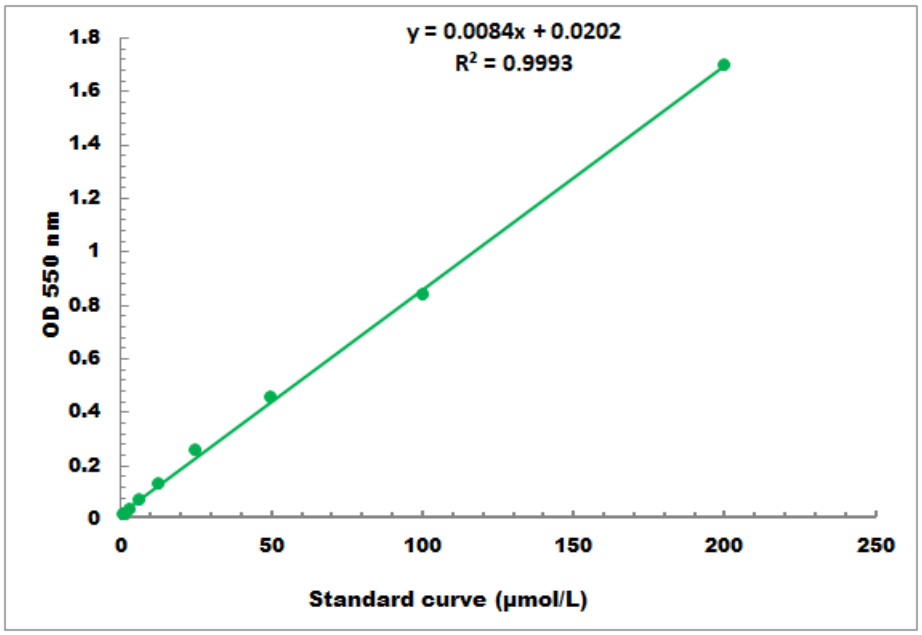
$V_{Sample}$: the volume of sample, 0.05 ml;

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

$V_{Assay}$: the volume of 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 - 200 μmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

## IX. NOTES