Nitric Oxide

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1063

(Version 1.5G)

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 I	20 ml x 1	4 °C
Assay Buffer II	20 ml x 1	4 °C
Dye Reagent A	Powder x 2	4 °C, keep in dark
Dye Reagent A Diluent	5 ml x 1	4 °C
Dye Reagent B	Powder x 2	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

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 for 1 month. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve.

Dye Reagent A: add 2.5 ml Dye Reagent A Diluent to each Dye Reagent A bottle before use, mix. If any undissolved substance, please use water bath heating to dissolve it. Store at 4°C for 1 week.

Dye Reagent B: add 2.5 ml distilled water to each Dye Reagent B bottle before use. Store at 4°C for 1 week.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 800 µl distilled water for 5×10^6 cells or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); then add 100 µl Assay Buffer I mix, and 100 µl Assay Buffer II mix again, centrifuged at 10,000 rpm 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 800 µl distilled water, transfer it into the centrifuge tube; then add 100 µl Assay Buffer I mix, and 100 µl Assay Buffer II mix again, centrifuged at 10,000 rpm for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For liquid samples

If the sample does not contain any protein or less protein, it can be assayed directly. If the sample contains more protein, the samples should be cleared by mixing 800 µl sample with 100 µl Assay Buffer I and 100 µl Assay Buffer II. Centrifuge 10 min at 10,000 rpm. Transfer the supernatant into a clean tube for detection (dilution factor n = 1.25).

Note: If Assay Buffer I and II were used for sample deproteinization, **Control** should be prepared parallelly by mixing 800 µl distilled water with 100 µl Assay Buffer I and 100 µl Assay Buffer II. Centrifuge 10 min at 10,000 rpm, take the supernatant as the control.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

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

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** 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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{Sample} - OD_{Control}) - Intercept}{Slope} \times n \quad (\mu mol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1. According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \times n \quad (\mu mol/ml)$$

2.2. According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{Assay})} \quad (\mu mol/g)$$

2.3. According to the quantity of cells or bacteria

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times (N \times V_{Sample} / V_{Assay})} \quad (\mu mol/10^4)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, μmol/ml

W: the weight of sample, g

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

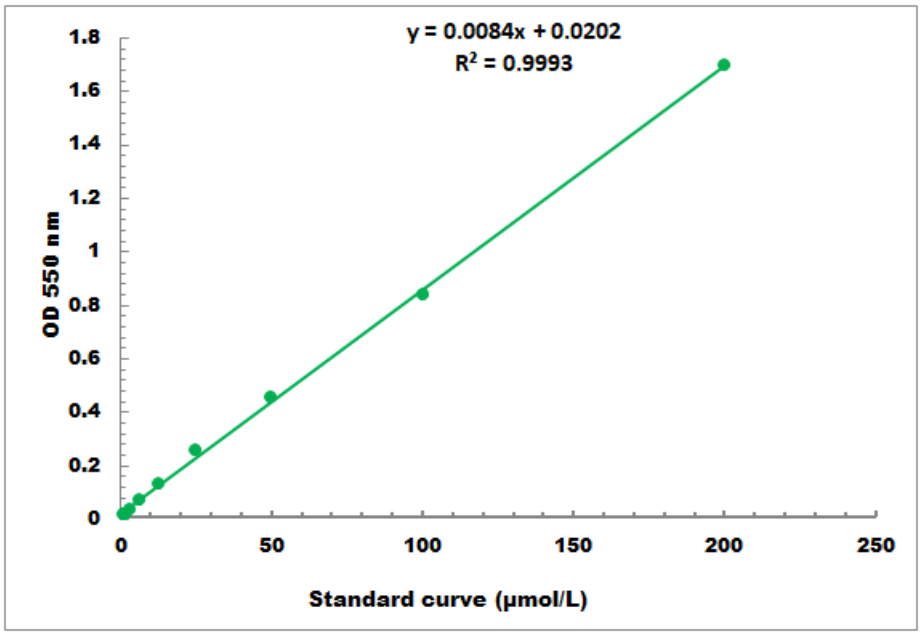
$V_{Standard}$: the volume of standard in assay procedure, mL

V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of distilled water, Assay Buffer I and Assay Buffer II, 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