Nitric Oxide (NO) Assay Kit

(Cat N.: BC004 Specification:96T Microplate method)

1. Significance and Principle of the Kit

NO is one kind of reactive free radicals in vivo, and functions as the second messenger and the neurotransmitter. However, it can act as an effector as well and because of this affect wide range of physiological reactions like vascular smooth muscle relaxation, platelet aggregation inhibition, cerebral blood flow regulation, cellular toxic effect and immunity modulation and atherosclerosis. Abnormality on NO production is closely related to development with diseases. Therefore researchers show increased interest in the NO related topics.

Blood NO is unstable with short half-life period and thus NO in blood is located at vascular endothelial cells, vascular smooth muscle cells, platelets and macrophages with the forms of nitrate and nitrite. It is proper to measure the nitrate and nitrite concentration in order to calculate the NO concentration.

NO is oxidized by oxygen to nitrate and nitrite which reacts with chromogenic agent to generate azo dyes and can be measured spectrophotometrically.

2. Components of the Kit (The kit is vaild for 6 months)

Reagent I: 1 Bottle X 20mL, preserved at 4°C

Reagent II: 1 Bottle X 10mL, preserved at 4°C

Reagent III: 1 Bottle X 10mL, preserved at 4°C without light. Warm the solution at 60°C to dissolve the crystal before use.

Reagent IV: 1 bottle X 3 mL, preserved at 4°C without light.

Reagent V: 1 bottle X 3 mL, preserved at 4°C.

Preparation of Chromogenic Agent: Blend reagent III, IV and V at the ratio of 2.5:1:1 before use.

2mM nitrite solution: 1 Vial X 1mL, preserved at 4°C.

Preparation of Standard Solution: Dilute 0.1mL 2mM nitrite solution with double distilled water (DDW) to 10mL.

3. Procedures

1. Pretreatment

Serum/ Plasma: 100μL serum is mixed with 200μL reagent I and then add 100μL reagent II. Mix thoroughly and set aside for 10 min. Centrifuge at 3500-4000 rpm for 15 min and take 160μL supernatant for measurement.

Tissue: 10% tissue homogenate should be centrifuged and 300μL supernatant is mixed with 200μL reagent I. Then add 100μL reagent II, mix thoroughly and set aside for 10 min. Centrifuge at 3500-4000 rpm for 15 min and take 160μL supernatant for measurement.

2. Measurement

	Blank	Standard	Sample
DDW (mL)	0.16		
Standard Solution (mL)		0.16	
Sample Supernatant (mL)			0.16
Chromogenic Agent (mL)	0.08	0.08	0.08
Mix thoroughly and set aside for 15 min. Record the optical density (OD) of each well at 550 nm			

4. Calculation

A. NO in serum

1. Formula

$$\frac{C_{NO}}{\mu M} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{20\mu M} \times 4(\text{Dilution Factor})$$

2. Example

0.1mL serum was measured with the OD values were 0.0406, 0.4117 and 0.0795 respectively.

$$\frac{C_{NO}}{\mu M} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{20\mu M} \times 4(\text{Dilution Factor})$$

$$= \frac{0.0795 - 0.0406}{0.4117 - 0.0406} \times 20 \times 4 = 8.38\mu M$$

B. NO in tissue

1. Formula

$$\frac{C_{NO}}{\mu mol/g\ prot} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{20\mu M} \times 2(\text{Dilution Factor}) \div \frac{C_{Prot}}{g/L}$$

2. Example

10% rat hepatic homogenate was prepared and 0.3mL homogenate was measured accordingly with the OD values of were 0.0406, 0.4117 and 0.0543 respectively. The protein concentration of the homogenate was 11.6574g/ L

$$\frac{C_{NO}}{\mu mol/g\ prot} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{20\mu M} \times 2(\text{Dilution Factor}) \div \frac{C_{Prot}}{g/L}$$

$$= \frac{0.0543 - 0.0406}{0.4117 - 0.0406} \times 20 \times 2 \div 11.6574 = 0.127\mu mol/g\ pro$$

5. Notes

1. Strictly obey the procedure mentioned above.
2. Supernatant should be clarified or otherwise mixture need to be re-centrifuged.
3. Hemolysis may interfere the results.
4. Serum and tissue samples can be preserved at -20 ° C for 1-2 months. The lower the temperature the longer the preservation period.

5. Culture medium measurement can be operated according to the serum measurement protocol.

6. Parameters

1. Detection Limit: 0.2μM
2. Coefficient of Variation within a batch: 2.11%
3. Coefficient of Variation among batches: 5.65%
4. Recovery: 99.5%
5. Linearity Range: 0.2-50μM with r2=0.999
6. Wavelength Range 530-570nm

Appendix Standard Curve Establishment

1. Pretreatment

Dilute the 2mM nitrite solution to the following concetrations: 0.05, 0.025, 0.02, 0.0125, 0.00625 and 0.003125mM.

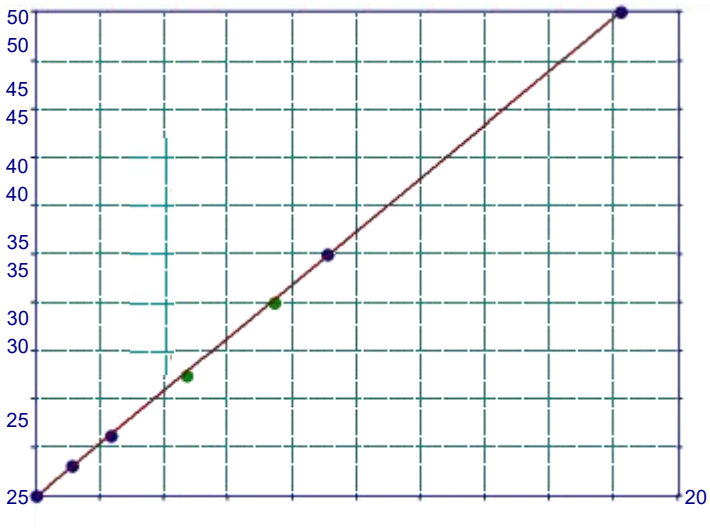
2. Measurement

	Blank	Standard
DDW (mL)	0.16	
Standard Solution (mL)		0.16
Chromogenic Agent (mL)	0.08	0.08
Mix thoroughly and set aside for 15 min. Record the OD of each well at 550 nm		

3. Results

Concentration (μM)	OD	ΔOD
0	0.0406	0
6.25	0.1581	0.1175
12.5	0.2766	0.2360
20	0.4117	0.3711
25	0.4957	0.4551
50	0.9523	0.9117

4. Standard Curve Concentration(μM)



OD Value