Nitric Oxide (NO) Assay Kit

Catalog No: SH0030
Method: Colorimetric method
Specification: 96T

Application

The kit applies to detect the concentration of NO in serum, plasma, nutrient solution, animal and plant tissue.

Detection significance

The half-life of NO is extremely short, it exists in form of nitrate or nitrite produced by vascular endothelial cell, vascular smooth muscle cell, platelet, and macrophage and so on. The concentration of NO can be indirectly measured by detecting that of nitrate or nitrite.

NO react with oxygen and water to generate nitrate or nitrite which can form a kind of pale red azo compound when meet with nitrate chromogenic reagent, the absorbance of the compound can be measured to calculate the concentration of NO indirectly.

Kit components

Item	Component	Specifications	Storage
Reagent 1	Sulphate solution	24 mL ×1 vial	4℃, 6 months
Reagent 2	Aqueous alkali	12 mL ×1 vial	4℃, 6 months
Reagent 3	Chromogenic agent A	3.8 mL ×1 vial	4℃, 6 months, shading light
	Note: If there is any crystal precipitation, please dissolve it fully with water bath at above 60℃ before use.		
Reagent 4	Chromogenic agent B	5.6 mg ×1 vial	4℃, 6 months, shading light
Preparation of reagent 4 working solution: Dissolve Chromogenic agent B fully with deionized water to 3.8 mL. The prepared solution can be stored at 4℃ for 2 months with shading light.			
Reagent 5	Acid solution	1.3 mL ×2 vials	4℃, 6 months
Preparation of chromogenic reagent: Prepare the reagent before use at the ratio of Reagent 3: Reagent 4 working solution: Reagent 5=3:3:2, it can be stored at 4℃ and can't be used when its color gets darker.			
Reagent 6	2 mmol/L Sodium nitrite standard solution	1 mL ×1 vial	4℃, 6 months

Experiment instruments

Tubes, micropipette, vortex mixer, microplate reader (550 nm)

Sample preparation

1. **Tissue homogenates:** Mince the tissues to small pieces, then be weighed and homogenized in homogenate medium (0.01M PBS, pH 7.4) on ice, the volume of PBS (mL): the weight of the tissue (g) =9:1. The tissue homogenate is centrifuged for 10 min at 3100 g and collect the supernatant for detect. Meanwhile, determine the concentration of supernatant (E-BC-K318, E-BC-K168, E-BC-K165).
2. **Serum (plasma):** detect directly.
3. **Cultured cells:**
   (1) Adherent cells should be detached with cell scraper or trypsin and then collect the cell sediment by centrifugation. (Suspension cells can be collected sediment by centrifugation directly).
   (2) Re-suspend the cells in cold PBS, centrifuge for 10 min at 1000 g and discard supernatant
   (3) Re-suspend cells in 1×PBS (It is recommended to add 0.4 mL PBS to 1×10^6 cells) then treat the cells with sonication on ice (power: 80W, 2 seconds/time, interval for 2 seconds, the total process will be 5 min). (or Freeze cells at ≤ -20℃. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times.)
   (4) The cell homogenate is centrifuged for 10 min at 3100 g and collect the supernatant for detect. Meanwhile, determine the concentration of supernatant (E-BC-K318, E-BC-K168, E-BC-K165).

Operation procedure

1. **Dilution of standard**

   Dilute 2 mmol/L standard sodium nitrite solution with deionized water to a serial concentration. The recommended dilution gradient is as follows: 300, 200, 150, 100, 40, 20, 10, 0 μmol/L.

2. **Pre-treatment**

	Standard tube	Sample tube
Different concentrations of sodium nitrite (μL)	A*	
Sample (μL)		A*
Reagent 1	200	200
Reagent 2	100	100
Mix fully and stand for 15 min at room temperature, centrifuge it at 3100 g for 10 min, take 160 μL of the supernatant for the following procedure.		

Note: a. For serum or plasma samples, A* is 200-300 μL.

b: For tissue or cell homogenates, A* is 100-300 μL.

3. Reaction

	Standard tube	Sample tube
Supernatant (μL)	160	160
Chromogenic reagent (μL)	80	80
Mix thoroughly for 2 min, stand for 15 min at room temperature, measure the OD of each well with micro-plate reader immediately at 550 nm wavelength.		

Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a standard curve with standard concentration on the x-axis and OD values on the y-axis. y = ax + b.

1. Tissue or cell homogenates:
   NO content (*μmol/gprot*) = (ΔA_{550} -b) ÷ a × f ÷ Cpr

2. Serum (plasma):
   NO content (*μmol/L*) = (ΔA_{550} -b) ÷ a × f

[Note]:

y: the absolute OD value of standard at 550 nm.

x: the concentration of standard.

a: the slope of standard curve.

b: the intercept of standard curve.

f: dilution factor of the sample before tested.

Cpr: the protein content of sample (gprot/L).

ΔA_{550}: the absolute OD value of sample, $\Delta A_{550}=OD_{sample}-OD_{blank}$

Technical parameter

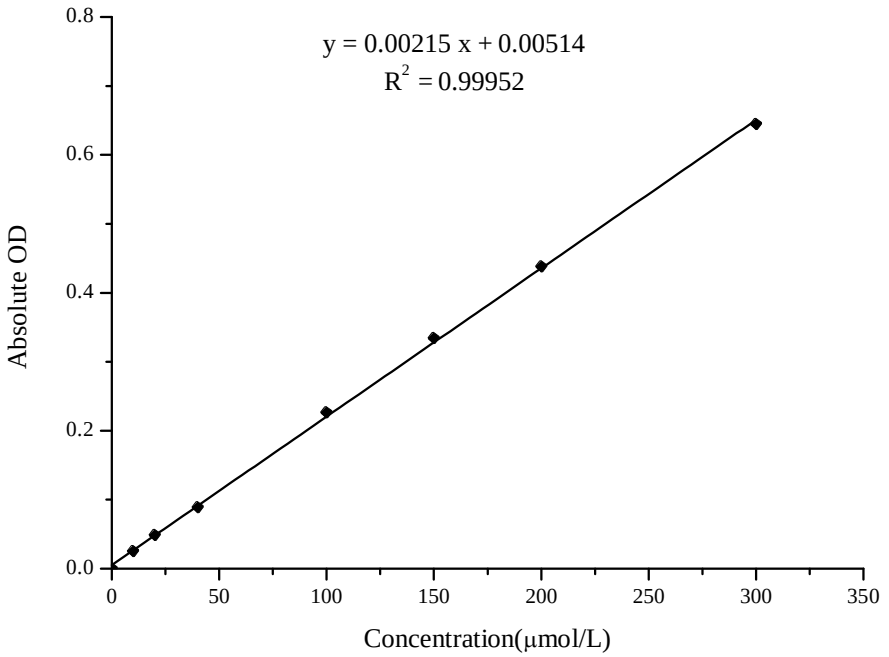
1. The sensitivity of the kit is 0.16 μmol/L.
2. The intra CV is 2.4% and the inter CV is 3.7%.
3. The recovery of the kit is 102%.
4. The detection range of the kit is 0.16~300 μmol/L.

Notes

1. Please progress strictly with operation procedures.
2. Use disposable EP tubes or clean glass tubes with stopper for centrifugation.
3. The supernatant for assay should not contain sediment, otherwise it will affect the results.
4. Samples can be stored at -20 ℃ for 1-2 months, the lower the temperature, the longer the storage time.
5. All reagents should be prepared the day before the assay, let it fully dissolved. Please add reagents to the bottom of well vertically and slowly, avoid to add on the wall of well and generate bubble.

Appendix: Standard curve

(This is for reference only)



This manual must be read attentively and completely before using this product.
May you have any problems, please contact our Technical Service Center for help.
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