Nitric Oxide (NO) Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC5485

Size:100T/96S

Components:

Extract solution: Liquid 110mL×1. Store at 2-8℃.

Reagent I: Powder×1. Store at 2-8℃. Add 6mL distilled water and dissolve completely at 50℃. Cool to room temperature before use. It could be stored at 2-8℃ for twelve weeks.

Chromogenic Liquid A: Liquid 6mL×1. Store at 2-8℃.

Chromogenic Liquid B: Liquid 6mL×1. Store at 2-8℃.

Chromogenic Liquid: Reagent IIA and Reagent IIB are mixed by the ratio of 50μL: 50μL (100μL, 1T) to make Reagent II according to sample number before use.

Standard: Liquid 1mL×1, 10μmol/mL sodium nitrite solution. Store at 2-8℃.

0.05μmol/mL standard solution: Mix 50μL 10μmol/mL sodium nitrite solution and 950μL distilled water to prepare a standard solution of 0.5μmol/mL; mix 100μL 0.5μmol/mL sodium nitrite solution and 900μL distilled water to prepare a standard solution of 0.05μmol/mL.

Product Description:

Nitric Oxide (NO) is a highly unstable biological free radical with small molecule, simple structure. NO is gas at room temperature, slightly soluble in water and fat-soluble, which can diffuse through biomembrane quickly. As a new biological messenger molecule, it plays a role in transmitting signals between cells and within cells. It is widely distributed in various tissues, especially neural tissues. It also plays a very important role in the nervous, circulatory, respiratory, digestive and urogenital systems of the body.

NO is easily oxidized to form NO_2^- in the body or in aqueous solution. Under acidic conditions, NO_2^- and Diazonium sulfonamide produce diazo compounds. The compounds could further couple with naphthyl vinyl diamine. The product has a characteristic absorption peak at 550 nm and its absorbance value can be measured to calculate the NO content.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, desk centrifuge, balance, transferpettor, micro glass cuvette/96 well plate, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Procedure:

I. Sample preparation

1. **Tissue:** According to the proportion of tissue weight (g): Extract solution volume (mL) of 2:5-10 to extract. It is suggested that 0.2 g of tissue with 1 mL of Extract solution and fully

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homogenized on ice bath. Centrifuge at 10000g for 15 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

2. **Bacteria/Cells:** Collect bacteria/cells into the centrifuge tube, after centrifugation discard supernatant. According to the proportion of bacteria/cells number (10^4): Extract solution volume (mL) of 1000-2000:1 to extract. It is suggested that add 1 mL of Extract solution to 10 million of bacteria/cells. Use ultrasonication to split bacteria/cells (place on ice, ultrasonic power 200W, working time 3 seconds, interval 7 seconds, repeat for 5 minutes). Centrifuge at 10000g for 15 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.
3. **Serum (plasma) or other liquid samples:** Detect directly. Centrifuge before detecting if there are precipitation in the samples.

II. Determination

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 550 nm and set spectrophotometer counter to zero with distilled water.
2. Add reagents in 1.5mL EP tube as the following:

Reagent (μL)	Test tube	Standard tube	Blank tube
Distilled water	-	-	100
Standard	-	100	-
Sample	100	-	-
Reagent I	50	50	50
Mix and react for 5min at room temperature. Centrifuge at 10000g for 5 minutes at 4℃ and take supernatant.			
Supernatant	100	100	100
Chromogenic Liquid	100	100	100
Mix and react for 10min at room temperature. Detect the absorbance value at 550 nm and record as A_T, A_B and A_S. $\Delta A_T=A_T-A_B$. $\Delta A_S=A_S-A_B$. Blank tube and standard tube need to test once or twice.			

III. NO content calculation:

1. Protein concentration:

   NO content (μmol/mg prot) $=\Delta A_T\times (C_S\div\Delta A_S)\times Vs\div(Vs\times Cpr)=0.05\times\Delta A_T\div\Delta A_S\div Cpr$
2. Sample weight:

   NO content (μmol/g weight) $=\Delta A_T\times (C_S\div\Delta A_S)\times Vs\div(W\times Vs\div V_T)=0.05\times\Delta A_T\div\Delta A_S\div W$
3. Bacteria/Cells:

   NO content (μmol/10^4 cell) $=\Delta A_T\times (C_S\div\Delta A_S)\times Vs\div(N\times Vs\div V_T)=0.05\times\Delta A_T\div\Delta A_S\div N$
4. Liqiud:

   NO content (μmol/mL) $=\Delta A_T\times (C_S\div\Delta A_S)\times Vs\div Vs=0.05\times\Delta A_T\div\Delta A_S$

   C_S: sodium nitrite concentration of standard solution, 0.05μmol/mL;

   Vs: Added sample supernatant volume, 0.1 mL;

V_T: Added Extract solution volume, 1mL;
Cpr: Sample protein concentration, mg/mL;
W: Sample weight, g;
N: Cell amount, 10^4 for one unit.

Note:

1. If ΔA_T is less than 0.005, it is recommended to increase added sample supernatant volume before determination. If ΔA_T is more than 0.7， it is recommended to dilute the sample with Extract solution before determination. And modify the calculation formula.
2. If sample supernatant has color (has absorption at 550nm), the control tubes of the sample need to be measured, that is, replace Reagent II with the same volume of distilled water. Detect the absorbance value at 550 nm and record as A_T, A_C, A_B and A_S. $\Delta A_T=A_T- A_C$. $\Delta A_S=A_S-A_B$. In this case the kit size is 100T/48S.

Experimental example:

1. Take 0.203g leaf of Albizia silk tree, add 1 mL of Extract solution, grind the homogenate with ice bath. Then operate according to the determination steps, calculate $\Delta A_T=A_T-A_B$=0.082-0.047=0.035, $\Delta A_S=A_S-A_B$=0.402-0.047=0.355. The result is calculated according to the sample weight:
   NO content (μmol/g weight) = $0.05\times\Delta A_T\div\Delta A_S\div W$ = 0.0243 μmol/g weight.
2. Take 0.215g mice heart, add 1 mL of Extract solution, grind the homogenate with ice bath. Then operate according to the determination steps, calculate $\Delta A_T=A_T-A_B$=0.102-0.047=0.055, $\Delta A_S=A_S-A_B$= 0.402-0.047=0.355. The result is calculated according to the sample weight:
   NO content (μmol/g weight) = $0.05\times\Delta A_T\div\Delta A_S\div W$ = 0.0360 μmol/g weight.
3. Take 100μL human serum and operate according to the determination steps, calculate $\Delta A_T=A_T-A_B$=0.071-0.047=0.024, $\Delta A_S=A_S-A_B$= 0.402-0.047=0.355. The result is calculated according to liquid volume:
   NO content (μmol/mL) =$0.05\times\Delta A_T\div\Delta A_S$ =0.0034 μmol/mL.

References：

[1] Green LC, Wagner DA, Glogowski J. et al. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids[J]. Analytical Biochemistry, 1982, 126(1): 131-138.

[2] Thomsen LL, Ching LM, Baguley BC. Evidence for the production of nitric oxide by activated macrophages treated with the antitumor agents flavone-8-acetic acid and xanthenone-4-acetic acid [J]. Animal Husbandry & Veterinary Medicine, 1990, 50(21): 6966-6970.

[3] Yang Wenping, Li Junmin, Wang Jinwen. Comparison of determination methods of serum nitric oxide content[J]. Experimental and Laboratory Medicine, 2002, 20(03): 147-148.

Related Products:

BC0080/BC0085	Nitrate reductase (NR) Activity Assay Kit
BC1480/BC1485	Soil/Water Nitrite Content Assay Kit
BC1490/BC1495	Food Nitrite Content Assay Kit
BC1470/BC1475	Nitric Oxide (NO) Content Assay Kit
BC2990/BC2995	Soil nitrate reductase (S-NiR) Activity Assay Kit

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