Nitric oxide (NO) Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: NA0510

Size: 100T/96S

Components:

Extract solution: 110 mL ×1, storage at 4℃.

Reagent 1: powder×1, storage at -20℃ and protected from light. Dissolve with 2.5 mL of distilled water before use, mix well, storage at -20℃ after packing.

Reagent 2: 3 mL×1, storage at 4℃.

Reagent 3A: 10 mL×1, storage at 4℃ and protected from light..

Reagent 3B: 10 mL×1, storage at 4℃ and protected from light. Before use, mix well according to Reagent 3A: Reagent 3B = 1:1 (V:V). Prepare according to sample number.

Standard: 1 mL ×1, 10 μmol/mL $NaNO_2$, storage at 4℃.

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 biofilms quickly. As a new type of 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^- and NO_3^- in the body or in aqueous solution. This method uses nitrate reductase to reduce NO_3^- to NO_2^- specifically. Under acidic conditions, NO_2^- and diazonium sulfonamide produce diazo compounds. The compounds could further coupled 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, low temperature centrifuge, water bath/constant temperature incubator, adjustable pipette, micro glass cuvette/ 96-well flat-bottom plate, mortar/homogenizer, ice and distilled water, EP tube.

Procedure:

I. Sample extraction:

1. Tissue: The mass (g): volume of Extract solution (mL)= 1:5 ~ 10, Suggest that weigh 0.2 g of sample, add 1 mL of Extract solution and homogenate in ice bath. Centrifuge at 4℃ and 12000 rpm for 15 minutes and discard precipitation, take the supernatant on ice for test.

2. Cells or bacteria: The ratio of cell number (10^4): volume of Extract solution(mL) 500-1000: 1, Collect 10 million bacteria or cells into a centrifuge tube, add 1 mL of Extract solution to ultrasonically break bacteria or cells (power 300W, ultrasonic 3s, 7s interval, total time 3 min). Centrifuge at 4℃ and 12000 rpm for 15 minutes and discard precipitation, take the supernatant on ice for test.
3. Liquid sample: direct determination

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader 30 min, adjust wavelength to550 nm, set zero with distilled water.
2. Keep Reagent 1 on ice.
3. Dilute the standard to 0.2、0.1、0.05、0.025、0.0125、0.00625、0.003125 μmol/mL with distilled water.
4. Sampling table (add the following reagents in a 0.6 mL EP tube)

Reagent (μL)	Blank Tube (Ab)	Test Tube (At)	Standard Tube (As)
Distilled water	120		20
Standard solution			100
Sample		100	
Reagent 1		20	
Mix and react for 60min at 37℃ water bath.			
Reagent 2	20	20	20
Mix and react for 5 min at room temperature. Centrifuge 3500 rpm for 10 minutes and take supernatant.			
Supernatant	100	100	100
Reagent 3	100	100	100

Vortex to mix, react for 10 minutes at room temperature, and measurement tube at 550 nm in micro glass cuvette/ 96-well flat-bottom plate, and record them as Ab, At, and As. Calculate ΔAt=At-Ab, ΔAs=As-Ab.

III. Calculation:

1. According to concentration of standard solution and absorbance to create the standard curve, take standard solution as X-axis, ΔAs as Y-axis. Take ΔA into the equation to obtain x (μmol/mL).
2. Calculation of NO content

(1) Protein concentration

NO (μmol/mg prot) = x ×Vs ÷(Cpr ×Vs)=x ÷Cpr

(2) Sample weight

NO (μmol/g fresh weight) = x ×Vs ÷(W ×Vs ÷Ve)=x ÷W

(3) The number of bacteria or cells:

NO（μmol /10^4 cell）= x ×Vs ÷(cell number ×Vs ÷Ve))=x ÷Number of cells

(4) Liquid volume:

NO (μmol/mL) = x ×Vs ÷Vs=x

Vs: sample volume, 0.1 ml;

Ve: extraction volume, 1 ml;

Cpr: sample protein concentration, mg/mL;

W: Sample weight, g

Number of cells: in units of 10^4.

Note:

1. When the At is higher than 1.2, it is recommended to test the sample after dilution and multiply it by the dilution factor in the calculation formula.
2. Try to use fresh samples for testing. Reagent 2 is corrosive. Please take protective measure during operation.
3. Tissue color has no effect on experimental results.
4. When the culture medium to be measured has color (has absorption at 550nm), you need to test the control tube of the sample, that is, replace the reagent 1 and the reagent 3 with the same volume of distilled water. At this time, the kit size is 100T/48S.
5. The calculation of ΔAt is "ΔAt=At-Ab" in the presence of Control tube.

Technical Specifications：

Minimum Detection Limit：0.00074 μmol/mL

Linear Range：0.00078-0.25 μmol/mL

Recent Product citations：

[1] Peng X, Zhu L, Guo J, et al. Enhancing biocompatibility and neuronal anti-inflammatory activity of polymyxin B through conjugation with gellan gum[J]. International journal of biological macromolecules, 2020, 147: 734-740.