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Nitric Oxide (NO) Content Assay Kit

Visible Spectrophotometry
Note: The reagents of this product have been changed. Please strictly follow this instruction.
Operation Equipment: Visible spectrophotometer
Cat No: AK0259-50T-48S
Size: 50T/48S

Product Composition:

Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact SunLong staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1 bottle	2-8℃
Reagent I	Powder ×1 tube	-20℃
Reagent II	Powder ×1 tube	-20℃
Reagent III	Powder ×2 tubes	-20℃
Reagent IV	Liquid 3 mL×1 bottle	2-8℃
Reagent V	Liquid 50 μL×1 tube	2-8℃
Chromogenic solution A	Liquid 15 mL×1 bottle	2-8℃
Chromogenic solution B	Liquid 15 mL×1 bottle	2-8℃
Clarifying agent	Powder ×1 bottle	2-8℃
Standard	Liquid 1 mL×1 tube	2-8℃

Solution Preparation:

1. Reagent I: before use, add 1.8 mL of distilled water; aliquot and store at -20℃ for 2 weeks; avoid repeated freezing and thawing.

2. Reagent II: before use, add 1 mL of distilled water; aliquot and store at -20℃ for 4 weeks; avoid repeated freezing and thawing.

3. Reagent II working solution: prepare according to the sample number at the ratio of Reagent II : distilled water = 10 μL : 590 μL (15T); use up on the same day.

4. Reagent III: before use, add 550 μL of distilled water to one tube; aliquot and store at -20℃ for 2 weeks; avoid repeated freezing and thawing. The apparent amount of powder may differ between tubes due to freeze-drying; this does not affect the use and the actual mass is the same.

5. Reagent V: prepare according to the sample number at the ratio of Reagent V (V) : distilled water (V) = 10 μL : 450 μL (11T); prepare just before use.

6. Chromogenic solution: mix chromogenic solution A : chromogenic solution B = 1 : 1 thoroughly before use; prepare just before use.

7. Clarifying agent: before use, add 15 mL of distilled water and dissolve by shaking or heating at 50℃. The solution is a saturated solution; take the supernatant for use, which can be stored at 2-8℃ for 12 weeks.

8. Standard: 10 μmol/mL sodium nitrite. Take 20 μL of the 10 μmol/mL standard and add 780 μL of distilled water to obtain 0.25 μmol/mL standard solution; then take 50 μL of the 0.25 μmol/mL standard solution and mix with 450 μL of distilled water to obtain 0.025 μmol/mL standard solution.

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Product Description:

Nitric oxide (NO) is a highly unstable, small, lipid-soluble free radical that is a gas at room temperature and slightly soluble in water. It diffuses rapidly across biological membranes and acts as a novel biological messenger between and within cells, and it plays important roles in the nervous, circulatory, respiratory, digestive and urogenital systems.

NO is easily oxidized to NO_2^- and NO_3^- in vivo or in aqueous solution. In this method, nitrate reductase specifically reduces NO_3^- to NO_2^-; under acidic conditions NO_2^- reacts with sulfanilamide to form a diazonium compound, which is further coupled with N-(1-naphthyl)-ethylenediamine to produce a red azo dye with a characteristic absorption peak at 550 nm, from which the NO content is calculated.

Reaction: NO → NO_3^-; NO_3^- + NADPH —(nitrate reductase)→ NO_2^- + $NADP^+$; NO_2^- + 4-aminobenzenesulfonamide —(H^+)→ diazonium salt; diazonium salt + N-(1-naphthyl)-ethylenediamine → red azo dye (550 nm)

Note: it is recommended to select 2-3 samples with large expected differences for a pre-experiment before the formal determination.

Reagents and Equipment Required but Not Provided:

Visible spectrophotometer, low-temperature centrifuge, analytical balance, water bath / constant-temperature incubator, 1 mL glass cuvette, adjustable pipettes, mortar / homogenizer / cell ultrasonic disrupter, ice and distilled water.

Operation Procedure:

I. Sample preparation (the sample size can be adjusted appropriately; the specific ratio can be referred to the literature.)

1. Tissue: add Extract solution at the ratio of mass (g) : volume (mL) = 1 : 5~10 (it is recommended to weigh about 0.2 g of sample and add 1.0 mL of Extract solution); homogenize in an ice bath, then centrifuge at 12000 rpm for 15 min at 4℃; take the supernatant and keep it on ice for testing.

2. Bacteria / cells: add Extract solution at the ratio of cell number (10^4) : volume (mL) = 500~1000 : 1 (it is recommended to add 10 million bacteria/cells to 1.0 mL of Extract solution); disrupt by ultrasonication in an ice bath (200 W, 3 s on, 7 s off, 5 min in total), then centrifuge at 12000 rpm for 15 min at 4℃; discard the pellet, take the supernatant and keep it on ice for testing.

3. Liquid sample: measure directly; if the liquid is turbid, centrifuge and take the supernatant for measurement.

II. Determination procedure:

1. Preheat the visible spectrophotometer for more than 30 min, adjust the wavelength to 550 nm and set zero with distilled water.

2. Operation table:

Reagent (μL)	Test tube	Standard tube	Blank tube
Sample	240	-	-
0.025 μmol/mL standard solution	-	240	-
Distilled water	-	160	400
Reagent I	20	-	-
Reagent II working solution	40	-	-

Reagent III	20	-	-
Mix well and react at 37℃ for 120 min	-	-	-
Reagent IV	40	-	-
Reagent V	40	-	-
Mix well and react at 37℃ for 30 min	-	-	-
Chromogenic solution	400	400	400
Mix well, stand at room temperature for 10 min, then measure the absorbance of each tube at 550 nm, recorded as A_{test}, $A_{standard}$ and A_{blank}. Calculate $\Delta A_{test} = A_{test} - A_{blank}$, $\Delta A_{standard} = A_{standard} - A_{blank}$. The blank tube and standard tube only need to be measured 1-2 times.			

III. Calculation of NO content:

1. By protein concentration: NO content (μmol/mg prot) = $\Delta A_{test} \times (C_{standard} \div \Delta A_{standard}) \times V_{sample} \div (V_{sample} \times Cpr) = 0.025 \times \Delta A_{test} \div \Delta A_{standard} \div Cpr$

2. By sample mass: NO content (μmol/g mass) = $\Delta A_{test} \times (C_{standard} \div \Delta A_{standard}) \times V_{sample} \div (W \times V_{sample} \div V_{total}) = 0.025 \times \Delta A_{test} \div \Delta A_{standard} \div W$

3. By cell / bacteria number: NO content (μmol/10^4 cell) = $\Delta A_{test} \times (C_{standard} \div \Delta A_{standard}) \times V_{sample} \div (V_{sample} \times N \div V_{total}) = 0.025 \times \Delta A_{test} \div \Delta A_{standard} \div N$

4. By liquid volume: NO content (μmol/mL) = $\Delta A_{test} \times (C_{standard} \div \Delta A_{standard}) \times V_{sample} \div V_{sample} = 0.025 \times \Delta A_{test} \div \Delta A_{standard}$

$C_{standard}$: concentration of the standard tube, 0.025 μmol/mL; V_{sample}: volume of sample added, 0.24 mL; V_{total}: volume of Extract solution added, 1 mL; Cpr: sample protein concentration, mg/mL; W: sample mass, g; N: total number of bacteria/cells, in 10^4.

Notes:

1. If the supernatant is still turbid after centrifugation, the reaction can be carried out directly; after the reaction, add 200 μL of Clarifying agent to the 0.8 mL reaction solution, mix and stand for 5 min, then centrifuge and take 1 mL of the supernatant for measurement; in this case the blank tube and the standard tube must be treated in the same way.

2. If ΔA_{test} is less than 0.01, increase the sample size and measure again; if ΔA_{test} is greater than 0.8, dilute the sample supernatant with Extract solution before measurement, and modify the calculation formula accordingly.

3. If the supernatant is colored (absorbs at 550 nm), a sample control tube is needed, in which the chromogenic solution is replaced by the same volume of distilled water; measure at 550 nm and record as $A_{standard}$, A_{test}, A_{blank} and $A_{control}$; then $\Delta A_{standard} = A_{standard} - A_{blank}$ and $\Delta A_{test} = A_{test} - A_{control}$. In this case the kit specification is 50T/24S.

Experimental Examples:

1. Take 0.107 g of Magnolia leaf sample, add 1 mL of Extract solution and homogenize in an ice bath; after centrifugation take the supernatant and follow the procedure. Measured with a 1 mL glass cuvette: ΔA_{test} = 0.205 - 0 = 0.205, $\Delta A_{standard}$ = 0.364 - 0 = 0.364. By sample mass: NO content = 0.025 × 0.205 ÷ 0.364 ÷ 0.107 = 0.132 μmol/g mass.

2. Take 0.0868 g of mouse heart sample and process it in the same way: ΔA_{test} = 0.212 - 0 = 0.212, $\Delta A_{standard}$ =

0.364 - 0 = 0.364. By sample mass: NO content = 0.025 × 0.212 ÷ 0.364 ÷ 0.0868 = 0.168 μmol/g mass.

3. Take 240 μL of bovine serum and follow the procedure: ΔA_{test} = 0.369 - 0 = 0.369, $\Delta A_{standard}$ = 0.364 - 0 = 0.364. By liquid volume: NO content = 0.025 × 0.369 ÷ 0.364 = 0.025 μmol/mL.