Nitric oxide (NO) Assay Kit(Enzymatic determination of total NO)

Note: The reagents have been changed, so please be aware of and follow this instruction strictly.

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: BC1475

Size: 100T/96S

Components:

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

Reagent I: powder×1, storage at -20℃. Dissolve with 1.8 mL of distilled water before use, mix well, storage at -20℃ for 4 weeks after packing.

Reagent II: powder×1, storage at -20℃. Dissolve with 1 mL of distilled water before use, mix well, storage at -20℃ for 4 weeks after packing.

Reagent II working solution: Before use, the reagent is prepared according to reagent II: distilled water = 10 μL: 590 μL (60T) according to the number of samples. the ratio of preparation. Use out on the same day.

Reagent III: powder×1, storage at -20℃. Dissolve with 0.55mL of distilled water before use, mix well, storage at -20℃ for 4 weeks after packing.

Reagent IV: 1.5 mL×1, storage at 4℃.

Reagent V: 25 μL×1. Before use, the Reagent V is prepared according to the ratio of Reagent V: distilled water = 5μL: 225μL (23T) according to the number of samples.

Developer A: 6mL×1, storage at 4℃.

Developer B: 6mL×1, storage at 4℃.

***Developer solution*:** Before use, according to the number of samples, the reagent is prepared according to the color Developer A: Developer B = 1: 1.

Clarifier: Powder×1. Before use, 6 mL distilled water is added, which could be shaken or heated at 50 °C to promote dissolution. This solution is a saturated solution, and the supernatant can be used at 2-8 °C for 12 weeks;

Standard: 1 mL ×1, 10 μmol/mL $NaNO_2$, storage at 4℃. Diluted with distilled water to 0.05 μmol / mL standard solution for test.

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 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, low temperature centrifuge, water bath/constant temperature incubator, adjustable pipette, micro glass cuvette/96-well 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 200W, ultrasonic 3s, 7s interval, total time 5 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. Sampling table

Reagent name（μL）	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	60	-	-
0.05μmol/mL Standard	-	60	-
Stilled water	-	40	100
Reagent I	5	-	-
Reagent II working solution	10	-	-
Reagent III	5	-	-
Mix well, react at 37 °C for 120 min		-	-
Reagent IV	10	-	-
Reagent V	10	-	-
Mix well, react at 37 °C for 30 min		-	-
Developer solution	100	100	100

After mixing, react at room temperature for 10 min, the absorbance values of each tube is measured at 550 nm, which are recorded as A_T, A_S and A_B, respectively. Calculate $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$. Blank tube and standard tube only need to measure 1-2 times.

III. Calculation:

1. Calculation of NO content

(1) Protein concentration

NO content（μmol/mg prot）= $\Delta A_T \times （C_S \div \Delta A_S） \times V_S \div (V_S \times Cpr) = 0.05 \times \Delta A_T \div \Delta A_S \div Cpr$

(2) Sample weight

NO content (μmol/g weight) = $\Delta A_T \times （C_S \div \Delta A_S） \times V_S \div (W \times V_S \div V_E) = 0.05 \times \Delta A_T \div \Delta A_S \div W$

(3) The number of bacteria or cells:

NO content（μmol /10^4 cell）= $\Delta A_T \times （C_S \div \Delta A_S） \times V_S \div (N \times V_S \div V_E) = 0.05 \times \Delta A_T \div \Delta A_S \div N$

(4) Liquid volume:

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

C_S: Standard tube concentration, 0.05μmol / mL

Vs: Sample volume, 0.06 ml;

Ve: Extraction volume, 1 ml;

Cpr: sample protein concentration, mg/mL;

N：Number of cells, 10^4 cells as a unit;

W：Sample weight, g’

Note:

1. If the supernatant of the sample homogenate is still turbid after centrifugation, the reaction can be carried out directly. After the reaction, 50μL clarifier is added to the 200μL reaction solution, and the mixture is allowed to stand for 5 min. After centrifugation, 200μL supernatant is taken for determination. In this case, the blank tube and the standard tube need to be treated the same.
2. If ΔA_T is less than 0.005 or A_T is close to the blank tube, the V_S can be increased before the determination; if the ΔA_T is greater than 0.6, it is recommended to dilute the sample supernatant with the extract before the determination. Note the simultaneous modification of the calculation formula.
3. If the sample supernatant has color (absorption peak at 550nm), it is necessary to make up the control tube of the sample (A_C), that is, the Developer solution is replaced by the same volume of distilled water. The absorbance A is measured at 550 nm, which is recorded as A_T, A_S, A_B and A_C, respectively. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. The kit specification is 100T / 48S.

Experimental examples:

1. Take 0.107 g magnolia leaf sample, add 1mL Extract solution to ice bath homogenate, centrifuge and take the supernatant, according to the measurement steps, measured and calculated with 96-well plate:

   $\Delta A_T = A_T - A_B = 0.172 - 0.045 = 0.127$, $\Delta A_S = A_S - A_B = 0.526 - 0.045 = 0.481$, according to the sample weight:

   The NO content (μmol/ g weight) = $0.05 \times \Delta A_T \div \Delta A_S \div W = 0.05 \times 0.127 \div 0.48 \div 0.107$
   $= 0.123$μmol / g mass.

2. Take 0.0868g mouse heart sample, add 1mL extract to ice bath homogenate, centrifuge and take the supernatant, operate according to the measurement steps, and measure and calculate with 96-well plate: $\Delta A_T = A_T - A_B = 0.187 - 0.045 = 0.142$, $\Delta A_S = A_S - A_B = 0.526 - 0.045 = 0.481$, calculated according to sample weight:

   The NO content (μmol /g weight) = $0.05 \times \Delta A_T \div \Delta A_S \div W = 0.05 \times 0.142 \div 0.481 \div 0.0868$
   $= 0.170$μmol /g weight.

3. Take 60μL bovine serum samples, according to the determination steps, measured and calculated with 96-well plates: $\Delta A_T = A_T - A_B = 0.326 - 0.045 = 0.281$, $\Delta A_S = A_S - A_B = 0.526 - 0.045 = 0.481$, calculated by liquid volume:

   NO content (μmol/mL) = $0.05 \times \Delta A_T \div \Delta A_S = 0.05 \times 0.281 \div 0.481 = 0.029$ μmol / mL.

Recent Product citations:

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.

Related Products:

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