Nitric Oxide Synthase (NOS) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC5680

Size:50T/48S

Components:

Extract solution I: Liquid 60mL×1. Store at -20°C.

Extract solution II: Liquid 0.6mL×2. Store at -20°C. It is volatile and sealed immediately after use.

Buffer solution: Liquid 30mL×1. Store at 2-8°C.

Reagent I: Liquid 8mL×1. Store at 2-8°C.

Reagent II: Powder×2. Store at -20°C. It is in the glass bottle. Add 6mL Buffer solution into one Reagent II. It can be stored at -20°C for 4 weeks after dispensing to avoid repeated freezing and thawing.

Reagent III: Liquid 50μL×1. Store at 2-8°C.

Reagent III working solution: Reagent III and Buffer solution are mixed by the ratio of 4μL: 396μL (400μL, 5T) to make Reagent III working solution according to sample number before use.

Reagent IV: Powder×2. Store at -20°C. Add 0.6mL Buffer solution into one Reagent IV before use. It can be stored at -20°C for 4 weeks after dispensing to avoid repeated freezing and thawing.

Reagent V: Powder×2. Store at -20°C. It is in the glass bottle. Add 2.4mL Buffer solution into one Reagent V. It can be stored at -20°C for 4 weeks after dispensing to avoid repeated freezing and thawing.

Working solution: Reagent I, Reagent II, Reagent III working solution, Reagent IV and Reagent V are mixed by the ratio of 0.6mL: 1.0mL: 0.4mL: 0.1mL: 0.4mL (2.5mL, 5T) to make Working solution according to sample number before use.

Reagent VI: Liquid 2.5mL×1. Store at 2-8°C.

Reagent VII: Liquid 55μL×1. Store at 2-8°C.

Reagent VII working solution: Reagent VII and Buffer solution are mixed by the ratio of 10μL: 450μL(0.46mL, about 11T) to make Reagent VII working solution according to sample number before use.

Chromogenic solution A: Liquid 15mL×1. Store at 2-8°C.

Chromogenic solution B: Liquid 15mL×1. Store at 2-8°C.

Chromogenic solution: Chromogenic solution A and Chromogenic solution B are mixed by the ratio of 1: 1 to make Chromogenic solution according to sample number before use.

Standard: Liquid 1mL×1, 10μmol/mL sodium nitrite solution. Store at 2-8°C. Mix 5μL 10μmol/mL sodium nitrite solution and 995μL distilled water to prepare a 0.05μmol/mL standard solution before use.

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Note: Reagent II, Reagent IV and Reagent V are lyophilized powder. The differences in the amount of these powders may seem to be large, but the actual qualities are the same. It does not affect the detection.

Product Description:

Nitric Oxide Synthase (NOS, EC 1.14.13.39) is a kind of enzyme that catalyzes the synthesis of NO from L-arginine in vivo. It mainly exists in vascular smooth muscle cells, macrophages, endothelial cells, nerve cells, liver cells, glomerular membrane cells and other cells. As a cell signal molecule, it plays a very important role in the nervous, immune and cardiovascular systems of the body.

NOS catalyzes L-arginine, molecular oxygen and NADPH to form NO and $NADP^+$. NO is easily oxidized to form NO_2^- and NO_3^- 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 NOS activity.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, centrifuge, balance, transferpettor, mortar/homogenizer/cell ultrasonic crusher, 1mL glass cuvette, ice and distilled water.

Procedure:

I. Sample preparation

1. **Tissue:** It is suggested that 0.2 g of tissue with 0.98 mL of Extract solution I and 0.02 mL of Extract solution II and fully homogenized on ice bath. Centrifuge at 12000g 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. It is suggested that add 0.98 mL of Extract solution I and 0.02 mL of Extract solution II 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 12000g for 15 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.
3. **Liquid samples:** Detect directly. Centrifuge before detecting if there are precipitation in the samples.

Note: Extract solution I and Extract solution II are mixed by the ratio of 0.98mL: 0.02mL to prepare according to sample number before use.

II. Determination

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

Reagent (μL)	Test tube	Standard tube	Blank tube

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Sample	240	-	-
Working solution	500	-	-
Mix and react at 37°C for 60min, bath at 100°C for 5min and cool to room temperature. Centrifuge at 11000g for 10 minutes at 4°C and take all supernatant.			
Supernatant	all supernatant	-	-

Reagent VI	40	-	-
Reagent VII working solution	40	-	-
Mix and react at 37°C for 30min.		-	-
Standard	-	240	-
Distilled water	-	580	820
Chromogenic solution	400	400	400
Mix and react for 10min at room temperature. Detect the absorbance value at 550 nm and record as A_T, A_S and A_B. $\Delta A_T=A_T-A_B$. $\Delta A_S=A_S-A_B$. Blank tube and standard tube need to test once or twice.			

III. NOS activity calculation:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes production of 1 nmol NO in the reaction system per minute every mg protein.

NOS activity (U/mg prot) $=(\Delta A_T\div\Delta A_S\times C_S)\times V_S\div(V_S\times Cpr)\times10^3\div T\times F=0.83\times\Delta A_T\div\Delta A_S\div Cpr\times F$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes production of 1 nmol NO in the reaction system per minute every g sample.

NOS activity (U/g weight) $=(\Delta A_T\div\Delta A_S\times C_S)\times V_S\div(W\times V_S\div V_T)\times10^3\div T\times F=0.83\times\Delta A_T\div\Delta A_S\div W\times F$

3. Bacteria/Cells number:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes production of 1 nmol NO in the reaction system per minute every 10^6 bacteria/cells.

NOS activity (U/10^6 cell) $=(\Delta A_T\div\Delta A_S\times C_S)\times V_S\div(N\times V_S\div V_T)\times10^3\div T\times F=0.83\times\Delta A_T\div\Delta A_S\div N\times F$

4. Liqiud volume:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes production of 1 nmol NO in the reaction system per minute every milliliter liqiud sample.

NOS activity (U/mL) =($\Delta A_T \div \Delta A_S \times C_S$)$\times Vs \div Vs \times 10^3 \div T \times F$ =$0.83 \times \Delta A_T \div \Delta A_S \times F$

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

Vs: Added sample supernatant volume, 0.24 mL;

V_T: Added volume of Extract solution I and Extract solution II, 1mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

N: Cell amount, 10^6 for one unit;

10^3: Unit conversion factor, 1μmol=10^3 nmol;

T: Reaction time, 60 min;

F: Dilution factor

Note:

1. NOS is not stable and easy to inactivate. It is recommended to use fresh samples for experiments and stored at -20℃ for fresh samples if not tested immediately.
2. The prepared Reagent II need to be stored at -20℃ after taking out the required volume according to the sample number.
3. If ΔA_T is less than 0.005, or A_T is closed to A_B, it is recommended to increase added sample supernatant volume or prolong time of the first reaction at 37℃ before determination. If ΔA_T is more than 0.4， it is recommended to dilute the sample with Buffer solution before determination. And modify the calculation formula.

Experimental example:

1. Take 0.2075g mice brain, add 0.98 mL of Extract solution I and 0.02 mL of Extract solution II, grind the homogenate with ice bath. Then operate according to the determination steps, calculate $\Delta A_T = A_T - A_B = 0.062 - 0.001 = 0.061$, $\Delta A_S = A_S - A_B = 0.386 - 0.001 = 0.385$. The result is calculated according to the sample weight:
   NOS activity (U/g weight) = $0.83 \times \Delta A_T \div \Delta A_S \div W$ = 0.634 U/g weight.
2. Take 0.5×10^6cell K562, add 0.98 mL of Extract solution I and 0.02 mL of Extract solution II, use ultrasonication to split it. Then operate according to the determination steps, calculate $\Delta A_T = A_T - A_B = 0.086 - 0.001 = 0.085$, $\Delta A_S = A_S - A_B = 0.386 - 0.001 = 0.385$. The result is calculated according to the cell number:
   NOS activity (U/10^6 cell) = $0.83 \times \Delta A_T \div \Delta A_S \div N$ = 0.366 U/10^6 cell.
3. Take 240μL horse serum and operate according to the determination steps, calculate $\Delta A_T = A_T - A_B = 0.043 - 0.001 = 0.042$, $\Delta A_S = A_S - A_B = 0.386 - 0.001 = 0.385$. The result is calculated according to liquid volume:
   NOS activity (U/mL) =$0.83 \times \Delta A_T \div \Delta A_S$ =0.091 U/mL.

References:

[1] List BM, Klösch B, Völker C. et al. Characterization of bovine endothelial nitric oxide

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synthase as a homodimer with down-regulated uncoupled NADPH oxidase activity: tetrahydrobiopterin binding kinetics and role of haem in dimerization[J]. Biochemical Journal, 1997, 323(1): 159-165.

[2] Dawson J, Knowles RG. A microtiter-plate assay of nitric oxide synthase activity[J]. Molecular Biotechnology, 1999, 12(3): 275-279.

[3] Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function[J]. European Heart Journal, 2012, 33(7): 829-837.

Related Products：

BC0080/BC0085	Nitrate reductase (NR) Activity Assay Kit
BC1470/BC1475	Nitric Oxide (NO) Content Assay Kit
BC5480/BC5485	Nitric Oxide (NO) Content Assay Kit
BC5690/BC5695	Nitric Oxide Synthase (NOS) Typed Activity Assay Kit