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/Microplate reader

Cat No: BC5685

Size: 100T/96S

Components:

Extract solution I: Liquid 110mL×1. Store at -20℃.

Extract solution II: Liquid 0.6mL×4. Store at -20℃. It is volatile and sealed immediately after use.

Buffer solution: Liquid 15mL×1. Store at 2-8℃.

Reagent I: Liquid 4mL×1. Store at 2-8℃.

Reagent II: Powder×1. Store at -20℃. It is in the glass bottle. Add 6mL Buffer solution before use. It can be stored at -20℃ for 4 weeks after dispensing to avoid repeated freezing and thawing.

Reagent III: Liquid 30μL×1. Store at 2-8℃.

Reagent III working solution: Reagent III and Buffer solution are mixed by the ratio of 2μL: 198μL (200μL, 10T) to make Reagent III working solution according to sample number before use.

Reagent IV: Powder×1. Store at -20℃. Add 0.6mL Buffer solution before use. It can be stored at -20℃ for 4 weeks after dispensing to avoid repeated freezing and thawing.

Reagent V: Powder×1. Store at -20℃. It is in the glass bottle. Add 2.4mL Buffer solution before use. It can be stored at -20℃ 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 0.3mL: 0.5mL: 0.2mL: 0.05mL: 0.2mL (1.25mL, 10T) to make Working solution according to sample number before use.

Reagent VI: Liquid 1.5mL×1. Store at 2-8℃.

Reagent VII: Liquid 30μL×1. Store at 2-8℃.

Reagent VII working solution: Reagent VII and Buffer solution are mixed by the ratio of 5μL: 225μL(0.23mL, about 23T) to make Reagent VII working solution according to sample number before use.

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

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

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℃. Mix 10μL 10μmol/mL sodium nitrite solution and 990μL distilled water to prepare a 0.1μmol/mL standard solution before use.

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

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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/Microplate reader, centrifuge, balance, transferpettor, mortar/homogenizer/cell ultrasonic crusher, micro glass cuvette/96 well plate, 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/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

Sample	60	-	-
Working solution	125	-	-
Mix and react at 37℃ for 60min, bath at 100℃ for 5min and cool to room temperature. Centrifuge at 11000g for 10 minutes at 4℃ and take all supernatant.			
Supernatant	all supernatant	-	-

Reagent VI	10	-	-
Reagent VII working solution	10	-	-
Mix and react at 37℃ for 30min.		-	-
Standard	-	60	-
Distilled water	-	145	205
Chromogenic solution	100	100	100
Mix and react for 10min at room temperature. Take 200μL reaction mixture, 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) $=(\Delta A_T\div\Delta A_S\times C_S)\times Vs\div(Vs\times Cpr)\times10^3\div T\times F=1.67\times\Delta A_T\div\Delta A_S\div Cpr\times F$

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) $=(\Delta A_T\div\Delta A_S\times C_S)\times Vs\div(W\times Vs\div V_T)\times10^3\div T\times F=1.67\times\Delta A_T\div\Delta A_S\div W\times F$

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) $=(\Delta A_T\div\Delta A_S\times C_S)\times Vs\div(N\times Vs\div V_T)\times10^3\div T\times F=1.67\times\Delta A_T\div\Delta A_S\div N\times F$

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) =($\Delta A_T \div \Delta A_S \times C_S$)×Vs÷Vs×$10^3$÷T×F =1.67×$\Delta A_T \div \Delta A_S$×F

C_S: sodium nitrite concentration of standard solution, 0.1μmol/mL;

Vs: Added sample supernatant volume, 0.06 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 0.5， 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 $\Delta A_T=A_T- A_B$=0.087-0.046=0.041, $\Delta A_S=A_S-A_B$=0.519-0.046=0.473. The result is calculated according to the sample weight:
   NOS activity (U/g weight) = 1.67×$\Delta A_T \div \Delta A_S \div$W = 0.698 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, extract by ultrasonication. Then operate according to the determination steps, calculate $\Delta A_T=A_T-A_B$=0.099- 0.046=0.053, $\Delta A_S=A_S-A_B$=0.519-0.046=0.473. The result is calculated according to cell number:
   NOS activity (U/10^6 cell) = 1.67×$\Delta A_T \div \Delta A_S \div$N = 0.374 U/$10^6$ cell.
3. Take 60μL horse serum and operate according to the determination steps, calculate $\Delta A_T=A_T-A_B$= 0.069-0.046=0.023, $\Delta A_S=A_S-A_B$=0.519-0.046=0.473. The result is calculated according to liquid volume:
   NOS activity (U/mL) =1.67×$\Delta A_T \div \Delta A_S$ =0.081 U/mL.

References：

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tetrahydrobiopterin binding kinetics and role of haem in dimerization[J]. Biochemical Journal, 1997, 323(1): 159-165.

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[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

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