(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Nitric Oxide Synthase (NOS) (Isoform) Activity Assay Kit

Catalog No.: BC00147

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Nitric Oxide Synthase (NOS) (Isoform) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Tissues, serum, plasma
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (optimal detection wavelength 530 nm)

Product Introduction

There are two main types of NOS: structural NOS (cNOS) and inducible NOS (iNOS). Structural NOS is primarily found in neurons and endothelial cells and is calcium-dependent; inducible NOS is primarily found in macrophages and is calcium-independent. This classification is based on this principle.

Principle

NOS catalyzes the reaction of L-Arg and molecular oxygen to produce NO. NO then reacts with nucleophiles to form colored compounds. The absorbance is measured at a wavelength of 530 nm, and the NOS activity can be calculated based on the absorbance.

Components

Components	Name	Size	Storage
Reagent 1	Substrate buffer	6 mL×4 bottles	-20℃, protected from light
Reagent 2	Accelerator	Powder×6 tubes	-20℃
		0.6 mL diluent×6 vials	-20℃

	(The powder dosage is small and may adhere to the tube wall or cap; it is not an empty tube. If it is not visible to the naked eye before use, centrifuge at 4000 rpm for 2 minutes before use.) Before testing, add 0.6 mL of the diluent to one tube of powder and mix thoroughly. After testing, the remaining reagent can be frozen at -20ºC or below for no more than one week. If the powder turns yellowish-brown or coffee-colored, it should not be used.		
Reagent 3	color developer	6 mL×2 bottles	4℃, protected from light
Reagent 4	Transparent agent	6 mL×2 bottles	RT
	It will solidify when it gets cold; it should be used only after it becomes clear in a 37°C water bath.		
Reagent 5	Terminator	6 mL×4 bottles	4℃
	It may appear cloudy when taken out of the refrigerator; heat to 37°C until clear before use.		
Reagent 6	Inhibitors	10 mL×1 bottle	4℃
	Please observe carefully before use. If crystals adhere to the bottle wall or bottom, shake the reagent and bottle together in hot water at 90-100℃ until they are completely dissolved. It can be used after cooling to room temperature.		

Note: For reagents stored at -20ºC, if multiple uses are required, please aliquot and store any excess reagents as soon as possible after the first thawing. Take out only the amount needed each time and thaw before use.

Storage

The kit has a shelf life of 6 months

Preparation before the experiment

Sample processing

1. Tissue preparation: Accurately weigh the tissue to be tested, and add 9 volumes of homogenizing medium (recommended: 0.9% physiological saline or 0.1 mol/L phosphate buffer with pH 7.0-7.4) at a weight (g): volume (mL) ratio of 1:9. Homogenize mechanically in an ice-water bath, centrifuge at 4000 rpm for 10 minutes, and collect the supernatant (10%

homogenate supernatant) for analysis. (For protein concentration determination, use a protein quantification kit BC00006.)

2. Liquid: direct measurement.

Note: Reagents should be removed from the refrigerator and allowed to stabilize at room temperature for 30 minutes before use. Reagents 4 and 5 must be clear and transparent before proceeding; otherwise, the results will be affected

Operation process

Reagents(μL)	TNOS Blank tube	TNOS Test tube	iNOS Blank tube	iNOS Test tube
Distilled water	a+100	100	a	--
Sample	--	a	--	a
Reagent 6	--	--	100	100
Shake the test tube rack.				
Reagent 1	200	200	200	200
Reagent 2	10	10	10	10
Reagent 3	100	100	100	100
Reagent 4	100	100	100	100
Reagent 5	2000	2000	2000	2000
Mix well, measure the absorbance of each tube at 530 nm with a 1 cm optical path, zero the tube with distilled water, and then measure the absorbance value.				

Note: 1. a is the reference sampling amount and the amount of double-distilled water to be replenished. 50-100 μL of 10% rat liver tissue, 30 μL of rat serum, 50 μL of 10% rat kidney homogenate, 30 μL of dog serum, and 100 μL of myocardial culture medium.

2. When performing colorimetric analysis, pay attention to whether there are crystals or turbidity in the tube. If so, place it in a 37°C water bath and stir gently a few times. After the turbidity or crystals disappear, perform colorimetric analysis.

Result calculation

1、Serum NOS enzyme activity calculation:

Unit definition: One unit of enzyme activity is defined as the amount of NO produced per milliliter of serum per minute.

$$\text{TNOS activity（U/mL）} = \frac{A_{\text{TNOS test}} - A_{\text{TNOS blank}}}{\varepsilon} \times \frac{V_{\text{reaction total}}}{V_{\text{sample}}} \times \frac{1}{d \times T} \div 1000$$

$$\text{iNOS activity（U/mL）} = \frac{A_{\text{iNOS test}} - A_{\text{iNOS blank}}}{\varepsilon} \times \frac{V_{\text{reaction total}}}{V_{\text{sample}}} \times \frac{1}{d \times T} \div 1000$$

2、Tissue NOS enzyme activity calculation:

Unit definition: One enzyme activity unit is defined as the amount of enzyme that produces 1 nmol of NO per minute from each milligram of tissue protein.

$$\text{TNOS activity（U/mgprot）} = \frac{A_{\text{TNOS test}} - A_{\text{TNOS blank}}}{\varepsilon} \times \frac{V_{\text{reaction total}}}{V_{\text{sample}}} \times \frac{1}{d \times T} \div Cpr$$

$$\text{iNOS activity（U/mgprot）} = \frac{A_{\text{iNOS test}} - A_{\text{iNOS blank}}}{\varepsilon} \times \frac{V_{\text{reaction total}}}{V_{\text{sample}}} \times \frac{1}{d \times T} \div Cpr$$

Annotation

V_{sample} : sample volume, mL;

$V_{reaction\ total}$: Total volume of the reaction solution, (a + 2.51) mL;

d : Colorimetric path length , cm ;

T : Reaction time , 15 min ;

ε: Extinction molar coefficient of the chromogenic substance , 38.3×10^{-6}L /mol/cm;

Cpr : Protein concentration in tissue homogenate , mgprot/L (prot refers to protein);

Normal reference value

Rat serum TNOS: 18.69±3.97 U/mL (n=45)

Rat kidney homogenate (TNOS): 0.536±0.134 U/mgprot (n=19)

Dog serum (TNOS): 20.57±3.39 U/mL (n=18)

Myocardial culture medium (TNOS): 0.698±0.110 U/mL (n=12)

Precautions

1. The accelerator is best prepared fresh and used within one day after preparation. If there is any leftover, it should be stored at -20ºC or below for no more than one week. Before preparation, both the accelerator and the diluent should be stored at -20ºC or below. If the pale yellow or white powder turns into coffee-colored or yellowish-brown granules, it is ineffective and cannot be used.
2. Reagent 6 is a supersaturated solution. If it is not used up in one experiment, it may crystallize when reused. Before use, it can be dissolved again by stirring with a glass rod in a boiling water bath.
3. NOS has low activity and poor stability. If the sample or homogenate supernatant is not to be tested immediately, it should be frozen at -20ºC or below.
4. Reagent 1 and the prepared Reagent 2 should avoid repeated freeze-thaw cycles as much as possible.
5. At low room temperature, NOS reagent 5 may become cloudy. If NOS reagent 5 has already been added to the test tube, hold the test tube up to a light source and observe for crystallization or turbidity. If crystallization or turbidity is present, place each test tube in a 37ºC water bath and shake for 5 minutes before colorimetric analysis.
6. Detection range: 0.2-81.9 U/mL.
7. This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.