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

Nitric Oxide Synthase (NOS) typed Assay Kit

Catalog No.: BC00193

Size: 50T

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) typed Assay Kit
Detection Method	Colorimetric
Sample Type	Serum, plasma, tissue
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (530 nm)

Principle

NOS catalyzes the reaction between L-Arg and molecular oxygen to produce nitric oxide (NO). NO reacts with nucleophilic substances to form colored compounds. The absorbance is measured at a wavelength of 530 nm, and NOS activity can be calculated according to the absorbance value. There are two main isoforms of NOS: constitutive NOS (cNOS) and inducible NOS (iNOS). Constitutive NOS (cNOS) is mainly distributed in neurons and endothelial cells and is calcium-dependent. Inducible NOS (iNOS) is primarily present in macrophages and is calcium-independent. NOS isoforms can be identified based on this principle.

Components

No.	Components	Size	Notes
Reagent 1	Substrate buffer	6 mL×2	Store at -20°C away from light.
Reagent 2	Accelerator	Powder ×3	Store at -20°C.
		Diluent 0.6 mL×3	Store at -20°C.
Reagent 3	Chromogenic reagent	6 mL×1	Store at 4°C away from light.
Reagent 4	Clearing agent	6 mL×1	Store at room temperature. It will solidify when cold. Use only after clarification in a 37°C water bath.

Reagent 5	Stop solution	60 mL×2	Store at 4°C. It may turn turbid when taken out of the refrigerator. Heat it at 37°C until clear before use.
Reagent 6	Inhibitor	10 mL×1	Store at 4°C. Please inspect carefully before use. If crystals adhere to the bottle wall or bottom, place the entire reagent bottle in hot water at 90-100°C, shake gently until the crystals are completely dissolved, cool to room temperature, and then the reagent is ready for use.

Notes:

The powder content in Reagent 2 is relatively small and may adhere to the tube wall or cap; this does not mean the tube is empty. If the powder is invisible to the naked eye during use, centrifuge the tube at 4000 rpm for 2 minutes before use. Before the test, take one vial of 0.6 mL diluent and add it to one powder vial, then mix thoroughly. The remaining reagent after testing can be stored frozen at below -20°C for no more than one week. Do not use the powder if it turns yellowish-brown or coffee-colored.

Reagents stored at -20°C, if repeated use is required, please aliquot the remaining reagent as soon as possible upon the first thaw and store separately. Take only the required amount each time, thaw it and use immediately.

Storage

The validity period of the kit is 6 months.

Preparation

Spectrophotometer with a detection wavelength of 530 nm and 1 cm path-length cuvettes, 37°C constant temperature water bath or air bath incubator, electric furnace (for reagent heating), tabletop low-speed centrifuge, pipettes of various specifications, double-distilled water, 0.9% normal saline or 0.1M PBS, vortex mixer, centrifuge tubes, and protein assay reagents (for tissue samples, available from our company).

- **Sample handling**

1. Tissue sample: Accurately weigh the tissue to be tested. Add homogenate medium at a weight (g) to volume (mL) ratio of 1:9 (0.9% normal saline or 0.1 mol/L phosphate buffer solution with pH 7.0–7.4 is recommended). Perform mechanical homogenization in an ice water bath, then centrifuge at 4000 rpm for 10 minutes. Collect the supernatant (10% tissue homogenate supernatant) for subsequent testing. (The protein concentration of the homogenate supernatant needs to be determined.)
2. Serum (plasma) samples: use directly.

Operation process

1. Take the reagents out of the refrigerator and let them equilibrate at room temperature for 30 minutes before use. Ensure Reagent IV and Reagent V are clear and transparent prior to operation; otherwise, the test results will be affected.

2. Operation Procedure

	TNOS Blank tube	**TNOS Assay tube**	**iNOS Blank tube**	**iNOS Assay tube**
Double-distilled water (μL)	a*+100	100	a*	
Sample (μL)		a*		a*
Reagent 6 (μL)			100	100
Mix thoroughly				
Reagent 1 (μL)	200	200	200	200
Reagent 2 (μL)	10	10	10	10
Reagent 3 (μL)	100	100	100	100
Reagent 4 (μL)	100	100	100	100
Reagent 5 (μL)	2000	2000	2000	2000
Mix well, set the wavelength to 530 nm with a 1 cm optical path, zero with distilled water, then measure the absorbance (A) of each tube.				

Notes:

a. a* refers to the reference sampling volume and the volume of supplemented double-distilled water. Use 50～100 μL of 10% rat liver tissue homogenate, 30 μL of rat serum, 50 μL of 10% rat kidney tissue homogenate, 30 μL of dog serum, and 100 μL of myocardial culture medium respectively.
b. Check whether there are crystals or turbidity in the tube before colorimetry. If present, place the tube in a 37°C constant temperature water bath and stir gently several times. Perform color determination only after the turbidity or crystals disappear completely.

Calculation

1. Calculation of Serum NOS Enzyme Activity

Unit Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol NO per minute per milliliter of serum.

$$\text{NOS Activity } (\text{U/mL}) = \frac{\mathbf{A_{Assay} - A_{Blank}}}{\varepsilon} \times \frac{V_{Total}}{V_{Sample}} \times \frac{1}{d \times T} \div 1000$$

2. Calculation of Tissue NOS Enzyme Activity

Unit Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol NO per minute per milligram of tissue protein.

$$\text{NOS Activity } (\text{U/mL}) = \frac{\mathbf{A_{Assay} - A_{Blank}}}{\varepsilon} \times \frac{V_{Total}}{V_{Sample}} \times \frac{1}{d \times T} \div Cpr$$

Annotation:

V_{Total}: Total volume of reaction system, (a*+2.51) mL

V_{Sample}: Volume of sample added, a* mL

d: Cuvette optical path, cm

T: Reaction time, 15 min

Cpr: Protein concentration of homogenate (supernatant), mg/mL

ε: Molar extinction coefficient of chromogen, 38.3×10^{-6}

Normal Reference Values

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. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. The accelerator is best prepared freshly for immediate use. It should be used up within one day after preparation. Any remaining portion shall be stored below -20°C for no more than one week. The undiluted Reagent 2 accelerator and diluent should both be stored below -20°C. The reagent is invalid and cannot be used if the pale yellow or white powder turns into coffee or tan particles.
4. Reagent 6 is a supersaturated solution. Crystals may form if the leftover solution is reused in subsequent experiments. Before use, dissolve the crystals by stirring with a glass rod in a boiling water bath.
5. NOS has low activity and poor stability. If the samples or homogenate supernatant cannot be detected immediately, they should be frozen and stored below -20°C.
6. Reagent 1 and prepared Reagent 2 shall avoid repeated freeze-thaw cycles as much as possible.
7. NOS Reagent 5 may turn turbid at low room temperature. If NOS Reagent 5 has already been added into the test tubes, inspect the tubes against light for crystals or turbidity. If crystals or

turbidity are observed, place each test tube in a 37°C water bath, shake for 5 minutes, and then proceed with colorimetry.

8. Detection range: 0.2–81.9 U/mL.