YK100　Human/Rat NO Synthase-1 EIA
Product Instructions

FOR LABORATORY USE ONLY

＜Distributed by＞

SCETI
3-6-7, Kasumigaseki, Chiyoda-ku, Tokyo 100-0013 Japan
URL: http://www.sceti.jp/export/　e-mail: exp-pet@sceti.co.jp
Tel: +81-3-5510-2347　Fax: +81-3-5510-0133

Contents

– **Please read all the package insert carefully before beginning the assay –**

YK100 Human/Rat NO Synthase-1 (NOS-I) EIA

Ⅰ. Introduction

Nitric Oxide syntahse-1 (NOS-1, nNOS) also known as brain NOS, is a constitutive nitric oxide synthase which presents not only in nerves, but also in different tissues such as macula densa, bronchial epithelium, pancreatic B-cells, mast cells, sketetal muscle, endothelium, and photoreceptors, etc. This enzyme immunoassay (EIA) kit (Cat. No. YK100) developed by Yanaihara Institute Inc. is a stable and convenient assay system for determination of the immunoreactive contents of NO synthase-1 in human/rat tissue extract and cell culture supernatants. The EIA kit is prepared by using synthetic human NOS-1 (998-1024) as standard and biotinylated human NOS-1 (998-1024) as labeled antigen. The kit contains specific polyclonal antibody to recognize human/rat NOS-1.

Ⅱ. Characteristics

This human/rat NO symthase-1 EIA (NOS-1) kit is used for direct quantitative determination of the immunoreactive contents of human/rat NOS-1 presented in tissue extracts and cell culture supernatant samples. It is different from other NOS kits to date, those are NOS activity assay kits through quantitativing nitric oxide production by the enzyme. The kit is characterized by its sensitive quantification and high specificity. In addition, it has no influences by other components in samples. Human NOS-1 (998-1024) standard used in this kit is a highly purified synthetic product (purity: higher than 98%). HPLC purified biotinylated glycylglycyl-human NOS-1 (998-1024) is used as labeled antigen.

< Specificity >

The EIA kit shows cross reactivity of 100% to human NOS-1 and rat NOS-1.

< Assay Principle >

This EIA kit for determination of immunoreactive contents of human/rat NOS-1 in tissue extract and cell culture supernatant samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to human/rat NOS-1 and biotin-avidin affinity system. The 96-wells plate is coated with goat anti rabbit IgG; NOS-1 standard or samples, labeled antigen and rabbit anti NOS-1 antibody are added to the wells for competitive immunoreaction. After incubation and plate washing, horseradish peroxidase labeled streptoavidin (SA-HRP) is added to form HRP labeled streptoavidin-biotinylated NOS-1-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by color reaction of TMB and the concentration of human/rat NOS-1 is calculated.

Ⅲ. Composition

Component	Form	Quantity	Main ingredient
1. Antibody Coated Plate	Microtiter plate	1 plate (96 wells)	Goat anti rabbit IgG
2. Standard	Lyophilized	1 vial (32.4pmol)	Synthetic human NOS-1 (998-1024)
3. Labeled Antigen	Lyophilized	1 vial	Biotinylated human NOS-1 (998-1024)
4. Specific Antibody	Liquid	1 bottle (6 mL)	Rabbit anti human NOS-1 (998-1024) IgG
5. SA-HRP Solution	Liquid	1 bottle (12mL)	HRP labeled streptoavidin
6. TMB Substrate	Liquid	1 bottle(12mL)	3,3'5,5'-tetramethyl benzidine (TMB)
7. Reaction Stopping Solution	Liquid	1 bottle (12mL)	1M H_2SO_4
8. Concentrated Wash Solution	Liquid	1 bottle (25 mL)	20 Fold concentrated saline
9. Buffer Solution	Liquid	1 bottle (25 mL)	BSA containing phosphate buffer
10. Adhesive Foil		3 pieces	

Ⅳ. Method

< Equipment required >

1. Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 450 nm
2. Washing device for microtiter plate and dispenser with aspiration system
3. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
4. Polypropylene made test tube for preparing standard solution
5. Graduated cylinder (500 mL)
6. Distilled water or deionized water

< Preparatory work >

Note: Standard and labeled antigen solutions should be used within 1 hour after being prepared.

1. Preparation of washing solution:
   Dilute 25 mL of Concentrated Wash Solution to 500 mL with distilled or deionized water.

2. Preparation of standard solution:
   Reconstitute Standard (lyophilized human NOS-1 (998-1024), 32.4pmol/vial) with 1mL of Buffer Solution, which affords 32.4 pmol/mL standard solution. The reconstituted standard solution 0.2 mL is diluted with 0.4 mL of Buffer Solution that yields 10.8 pmol/mL standard solution. Repeat the same dilution procedure to make 3.6, 1.2, 0.4 and 0.133 pmol/mL. Buffer Solution is used as 0 pmol/mL.

3. Preparation of labeled antigen solution:
   Reconstitute Labeled Antigen with 12 mL of Buffer Solution.

4. Other reagents are ready for use.

< Procedure >

1. Before starting the assay, bring all the reagents except samples to room temperature (20-30°C).

2. Add 0.35mL/well of washing solution into each of the wells and then aspirate it. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it firmly onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual wash solution.

3. Fill 100µL of labeled antigen solution into the wells first, then introduce 50µL of each of standard solutions or samples and finally add 50µL of specific antibody solution into wells.

4. Cover the plate with adhesive foil and incubate it at room temperature overnight (18-20 hr.)

5. Take off the adhesive foil, aspirate the solution in the wells and add 0.35mL/well of washing solution into each of the wells and then aspirate it. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it firmly onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual wash solution.

6. Pipette 100µL of SA-HRP solution into the wells.

7. Cover the plate with adhesive foil and incubate it at room temperature for 1 hour.

8. Take off the adhesive foil, aspirate and wash the wells 5 times with approximately 0.35 mL/well of washing solution. Finally, invert the plate and tap it firmly onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual wash solution.

9. Add 100μL of TMB Substrate into the wells, cover the plate with adhesive foil and keep it for 30 minutes at room temperature in a dark place for color reaction.

10. Add 100μL of reaction Stopping Solution into the wells to stop color reaction.

11. Read the optical absorbance of the solution in the wells at 450 nm. Prepare a standard curve on semilogarithmic graph paper by plotting B/Bo% or optical density on the ordinate against concentrations of standard solutions on the abscissa. (abscissa: concentration of standard; ordinate: B/B_0% or optical density). Calculate B/Bo% for each unknown sample and read values directly from the curve in pmol/mL. If a 4-parameter calibration curve fitting software be used, sample values may be easily calculated using the following formula: Y= (a-d)/(1+(x/c)^b)+d.
    Y; binding rate% or optical density X; concentration (pmol/mL) a,b,c,d; constant parameter

Ⅴ. Notes

1. 10~20 Fold of 10mM phosphate buffer (pH7.4) may be used to extract tissues. It is recommended that the extracting solution should be added with enzyme inhibitors such as aprotinin and PMSF, e.g.. Extracted tissue /culture supernatant solution should be adjusted its pH to about 7.0~7.4 before assay. If a low level concentration of tissue/culture extract sample is predicted, lyophilizing the supernatant, then re-dissolved the lyophilysate with kit Buffer Solution before assay. Extract sample should be kept below -30°C and avoid repeated freezing and thawing of samples.

2. Standard and labeled antigen solutions should be used with 1 hour after being prepared.

3. Pipetting operations may affect the precision of the assay, pipette standard solutions or samples into each well of the plate precisely. In addition, use clean test tubes or vessels in assay and a new tip for each standard diluting process and for each sample or standard solution pipetting to avoid cross contamination.

4. The kit can be used for twice separately. In that case, reconstituted reagents (standard and labeled antigen solutions) should be stored below -30°C and the other parts of kit stored at 4°C separately.

5. Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

6. Perform all the determination in duplicate.

7. When sample value exceeds 32.4 pmol/mL, it needs to be diluted with kit Buffer Solution to a proper concentration, and to be assayed again.

8. Color reaction should be carried out under the light proof condition.

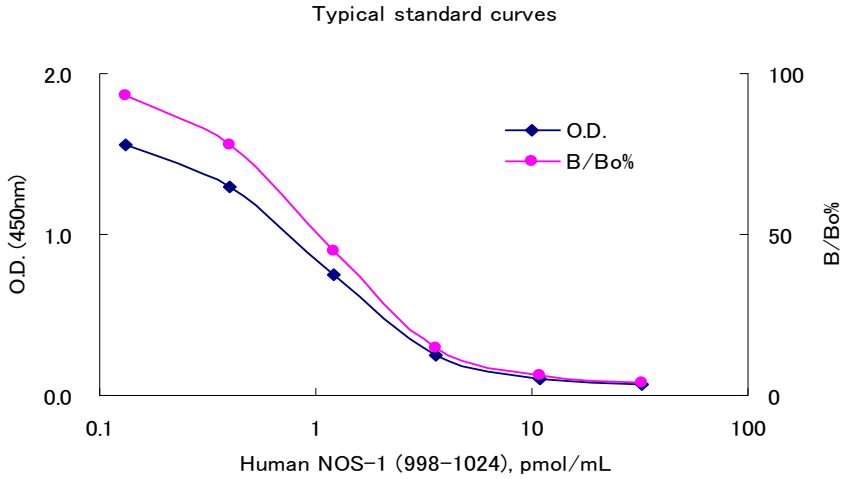
9. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

10. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

11. To quantitate accurately, always run a standard curve when testing samples.

Ⅵ. Performance Characteristics

<Assay range> 0.133-32.4pmol/mL



< Precision and reproducibility >

- Intra-assay CV(%): 4.0-5.3
- Inter-assay CV(%): 4.7-8.0

< Analytical recovery >

Extracted Sample	Human NOS-I added (pmol/mL)	Observed (pmol/mL)	Expected (pmol/mL)	Recovery (%)
Rat cerebellum	0.00	0.30	-	-
	0.51	0.97	0.81	120.1
	2.03	2.29	2.33	98.5
	8.11	8.65	8.41	102.8
Rat colon No. 1	0.00	0.73	-	-
	0.51	1.24	1.24	100.0
	2.03	2.74	2.76	98.8
	8.11	8.04	8.84	90.9
Rat colon No. 2	0.00	3.47	-	-
	0.51	4.76	3.98	119.8
	2.03	6.35	5.5	115.5
	8.11	13.09	11.58	113.0

<Dilution Test>

Sample*	Undiluted	1/2	1/4
Rat cerebellum A	21.25	20.62	20.8
Rat cerebellum B	21.77	20.90	21.73
Rat colon A	20.26	19.26	20.57
Rat colon B	24.60	22.42	23.37

* : Human NOS-I (998-1024) added samples, pmol/mL

Ⅶ. Stability and Storage

< Storage > Store all of the components at 2-8°C.

< Shelf life > The kit is stable under the condition for 24 months from the date of manufacturing. The expiry date is indicated on the label of the kit.

< Package > For 96 tests per one kit including standards

Ⅷ. References

1. Nakane M, et al (1993): Cloned human brain nitric oxide synthase is highly expressed in skeletal muscle. *FEBS* **316**, 175-180
2. Imai T, et al (1992): Expression of brain nitric oxide synthase mRNA in various tissues and cultured cells of rat. *Biomed Res* **13**, 371-374
3. Schmidt HHHW, et al (1994): Biochemistry and regulation of nitric oxide synthase, *Taniguchi Symposium on Brain Sciences* No.**17**, 3-18

<Manufacturer>
Yanaihara Institute Inc.

Updated on June 2nd, 2010