COSMO BIO CO., LTD.
Inspiration for Life Science

Human/Rat NO Synthase-I EIA

Product Instructions

Cat. No. YII-YK100-EX

FOR LABORATORY USE ONLY

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Contents

Attention:

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

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

Ⅰ. Introduction

This enzyme immunoassay (EIA) kit is a stable and convenient assay system for NO synthase-I (NOS-I) in human/rat tissue extract. The EIA kit is prepared by using synthetic NOS-I (998-1024) as standard and biotinylated NOS-I (998-1024) as labeled antigen. The kit contains specific polyclonal antibody to recognize human/rat NOS-I.

YK100 Human/Rat NO Synthase-I EIA Kit

- ▼ The assay kit can measure human/rat NOS-I in the range of 0.133-32.4 pmol/mL
- ▼ The assay completed within 16-20 hr + 1.5 hr.
- ▼ With one assay kit, 41 samples can be measured in duplicate
- ▼ Test sample: human and rat tissue extract
  Sample volume: 50 µL
- ▼ The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV (%): 4.0-5.3
  Inter-assay CV (%): 4.7-8.0
- ▼ Stability and Storage

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

  The kit is stable under the condition for 12 months from the date of manufacturing. The expiry date is indicated on the label of the kit.

Contents

1) Antibody coated plate
2) NOS-I Standard
3) Labeled antigen
4) Specific antibody
5) SA-HRP solution
6) Substrate sulotion
7) Stopping solution
8) Washing solution (concentrated)
9) Buffer solution
10) Adhesive foil

Ⅱ. Characteristics

This EIA kit is used for quantitative determination of human/rat NOS-I in tissue extract samples. The kit is characterized by its sensitive quantification and high specificity. In addition, it has no influences by other components in samples. Human NOS-I (998-1024) standard of this kit is a highly purified synthetic product (purity: higher than 98%). HPLC purified biotinylated glycylglycyl-human NOS-I (998-1024) is used as labeled antigen.

< Specificity >

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

< Assay Principle >

This EIA kit for determination of human/rat NOS-I in tissue extract samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to human/rat NOS-I and biotin-avidin affinity system. The 96-wells plate is coated with goat anti rabbit IgG, NOS-I standard or samples, labeled antigen and rabbit anti NOS-I antibody are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP labeled streptoavidin (SA-HRP) is added to form HRP labeled streptoavidin-biotinylated NOS-I-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-I is calculated.

Ⅲ. Composition

Component	Form	Quantity	Main ingredient
1. Antibody coated plate	MTP*1	1 plate (96 wells)	Goat anti rabbit IgG
2. NOS-I Standard	Lyophilized	1 vial	Synthetic human NOS-I (998-1024) (32.4pmol)
3. Labeled antigen	Lyophilized	1 vial	Biotinylated human NOS-I (998-1024)
4. Specific antibody	Liquid	1 bottle (6 mL)	Rabbit anti human NOS-I (998-1024) IgG
5. SA-HRP solution	Liquid	1 bottle (12mL)	HRP labeled streptoavidin
6. Substrate solution	Liquid	1 bottle(12mL)	3,3'5,5'-tetramethyl benzidine (TMB)
7. Stopping solution	Liquid	1 bottle (12mL)	1M H_2SO_4
8. Washing solution (concentrated)	Liquid	1 bottle (25 mL)	Concentrated saline
9. Buffer solution	Liquid	1 bottle (25 mL)	Phosphate buffer
10. Adhesive foil		3 pieces	

*1 MTP……Microtiter plate

Ⅳ. Method

< Equipment required >

1. Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 490nm
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 antigen and labeled antigen solutions should be prepared with 1 hour of being use.

1. Preparation of washing solution:
   Dilute 25 mL of washing solution (concentrated) to 500 mL with distilled or deionized water.
2. Preparation of standard solution:
   Reconstitute NOS-I standard (lyophilized human NOS-I 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:
   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 and samples to room temperature at least for 1 hour.

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).

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 (16-20 hr.)

5. Take off the adhesive foil, aspirate the solution in the wells and wash the wells 3 times with approximately 0.35 mL/well of washing 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.

9. Add 100µL of substrate solution 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 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 concentration of NOS-I 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 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% 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. Extracted tissue supernatant solution should be adjusted its pH to about 7.0~7.4. It is recommended that the extracting solution should be added with enzyme inhibitors such as aprotinin and PMSF, e.g. and tissue extract supernatant be lyophilized first, and then re-dissolved with kit buffer solution before assay. Extract sample should be kept below -30°C and avoid repeated freezing and thawing of samples.

2. Standard antigen and labeled antigen solutions should be prepared with 1 hour of being use.

3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2-8°C.

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

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

6. When sample value exceeds 32.4 pmol/mL, it needs to be diluted with buffer solution to a proper concentration, and to be assayed again.

7. Perform all the determination in duplicate.

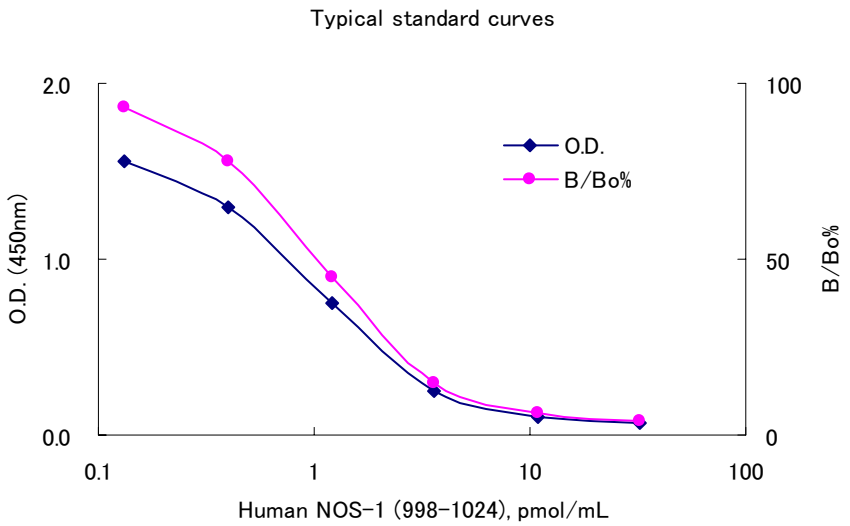
8. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

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

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

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

Ⅵ. Performance Characteristics



< Precision and reproducibility >

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

< Assay range > 0.133-32.4 pmol/mL

< 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＞

Dilution*	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 12 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

Manufactured by Yanaihara Institute Inc.

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