Human/Rat NO Synthase-I ELISA

Cat. No.: RSCYK100R

1. 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.

RSCYK100R Human/Rat NO Synthase-I EIA Kit

- ▼ The assay kit can measure human/rat tissue extract in the range of 0.412 - 100 ng/mL
- ▼ The assay completes within 16-18 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 concisted 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 ℃.
  The kit is stable under the condition for 6 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) OPD tablet
7) Substrate buffer
8) Stopping solution
9) Washing solution (concentrated)
10) Buffer solution
11) Adhesive foil

2. 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 standard (998-1024) 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 o-phenylenediamine dihydrochloride (OPD) and the concentration of human/rat NOS-I is calculated.

3. Composition

	Component	Form	Quantity	Main ingredient
①	Antibody coated plate	Microtitier plate	1 plate (96 wells)	Goat anti rabbit IgG
②	NOS.I standard	Lyophilized	1 vial	Synthetic human NOS-I (998-1024) (100 ng)
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 IgG
⑤	SA-HRP solution	Liquid	1 bottle (22 mL)	HRP labeled streptoavidin
⑥	OPD tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
⑦	Substrate buffer	Liquid	1 bottle (25 mL)	0.015% Hydrogen Peroxide
⑧	Stopping solution	Liquid	1 bottle (6 mL)	1M H_2SO_4
⑨	Washing solution (concentrated)	Liquid	1 bottle (25 mL)	Concentrated saline
⑩	Buffer solution	Liquid	1 bottle (25 mL)	Phosphate buffer
⑪	Adhesive foil		3 pieces	

4. Method

Equipment required

1. Photometer for microtitration plate (plate reader),which can read extinction 2.5 at 490 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 (1,000 mL)

6. Distilled water or deionized water

Preparatory work

1. Preparation of standard solution:
   Reconstitute NOS-I standard (lyophilized human NOS-I 100 ng/vial) with 1mL of buffer solution, which affords 100 ng/mL standard solution. The reconstituted standard solution 0.2 mL is diluted with 0.4 mL of buffer solution that yields 33.33 ng/mL standard solution. Repeat the same dilution procedure to make 11.11, 3.703, 1.235 and 0.412 ng/mL. Buffer solution is used as 0 ng/mL.

2. Preparation of labeled antigen:
   Reconstitute labeled antigen with 12 mL of buffer solution.

3. Preparation of substrate solution:
   Resolve 2 OPD tablets with 22 mL of substrate buffer. If the kit is used twice separately, it should be resolve 1 OPD tablet with 11mL of substrate buffer. This procedure should be prepared immediately before use.

4. Preparation of washing solution:
   Dilute 25 mL of washing solution (concentrated) to 500 mL with distilled or deionized water.

5. Other reagents are ready for use.

Procedure

1. Before starting the assay, bring all the reagents and samples to room temperature (20～30℃) 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 (0, 0.412, 1.235, 3.703, 11.11 and 33.33 ng/mL) 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 200μL 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 200μL of substrate solution into the wells, cover the plate with adhesive foil and waiting color reaction for 30 minutes at room temperature.

10. Add 50μL of stopping solution into the wells to stop color reaction.

11. Read the optical absorbance of the solution in the wells at 490 nm. Prepare a standard curve on semilogarithmic graph paper by plotting B/Bo% on the ordinate against concentration of NOS-I on the abscissa. (abscissa: concentration of standard; ordinate: B/B_0%). Calculate B/Bo% for each unknown sample and read values off the curve in ng/mL.

5. Notes

1. Extract sample should be kept below -30°C and avoid repeated freezing and thawing of samples. It is recommended that tissue extracts have better to be lyophilized first, and then dissolved with buffer solution before assay.

2. Standard, labeled antigen, substrate solution should be prepared immediately before 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 if be used within one week.

6. When sample value exceeds 100 ng/mL, it needs to be diluted with buffer solution to a proper concentration.

7. Perform all the determination in duplicate.

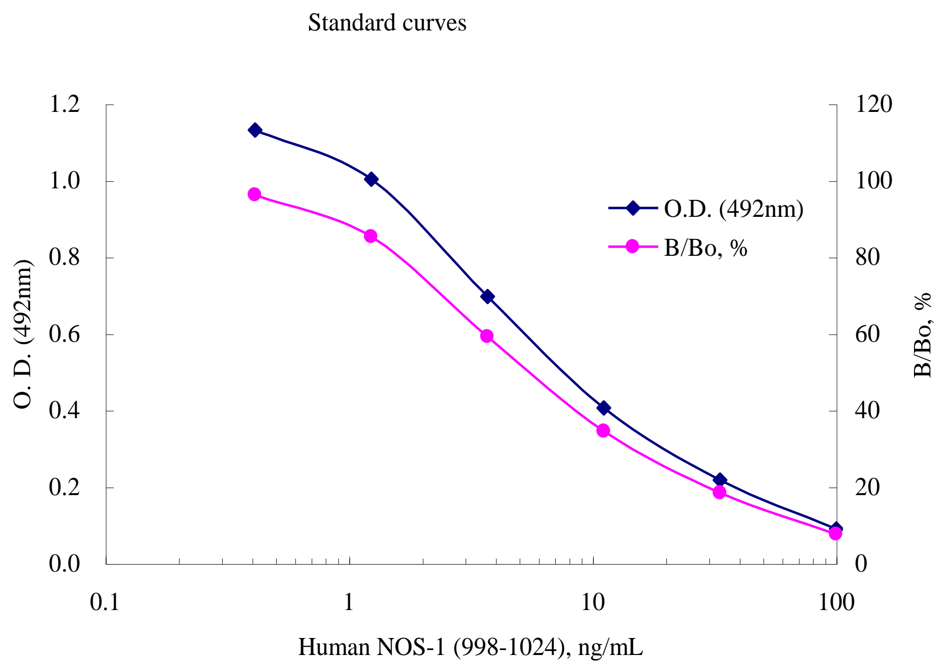
8. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

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.

6. Performance Characteristics



Precision and reproducibility

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

Assay range 0.412-100 ng/mL

Analytical recovery

Extracted Sample	Human NOS-I added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Rat cerebellum	0.00	0.93	-	-
	1.56	2.99	2.49	120.1
	6.25	7.07	7.18	98.5
	25.0	26.65	25.93	102.8
Rat colon No. 1	0.00	2.25	-	-
	1.56	3.81	3.81	100.0
	6.25	8.45	8.50	98.8
	25.0	24.78	27.25	90.9
Rat colon No. 2	0.00	10.69	-	-
	1.56	14.68	12.25	119.8
	6.25	19.57	16.94	115.5
	25.0	40.34	35.69	113.0

Dilution

Dilution*	Undiluted	1/2	1/4
Rat cerebellum A	65.48	63.54	64.08
Rat cerebellum B	67.08	64.40	66.97
Rat colon A	62.44	59.34	63.38
Rat colon B	75.80	69.10	72.02

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

7. Stability and Storage

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

Shelf life The kit is stable under the condition for 6 months from the date of manufacturing

The expiry date is indicated on the label of the kit.

Package For 96 tests per 1 kit including standards

8. 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

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