KAMIYA BIOMEDICAL COMPANY

Human and Rat NO Synthase-I EIA

For the quantitative determination of NO Synthase-I in human or rat tissue extracts.

Cat. No. KT-371

For Research Use Only.

PRODUCT INFORMATION

Human and Rat NO Synthase-I EIA
Cat. No. KT-371

INTENDED USE
For the quantitative determination of NO Synthase-I in human or rat tissue extracts. For research use only.

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 calibrator and biotinylated NOS-I (998-1024) as labeled antigen. The kit contains specific polyclonal antibody to recognize human/rat NOS-I. 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) calibrator 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.

PRINCIPLE
This kit is based on a competitive enzyme immunoassay using a combination of a highly specific antibody to human/rat NOS-I and a biotin-avidin affinity system. The 96-well plate is coated with goat anti-rabbit IgG, NOS-I Calibrators 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 the color reaction of TMB and the concentration of human/rat NOS-I is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	MTP[*1]	1 plate (96-well)	Goat anti-rabbit IgG
2. NOS-I Calibrator	Lyophilized	1 vial (32.4 pmol)	Synthetic human NOS-I (998-1024)
3. Labeled Antigen	Lyophilized	1 vial	Biotinylated human NOS-I (998-1024)
4. NOS-I Antibody	Liquid	1 bottle (6 mL)	Rabbit anti-human NOS-I (998-1024) IgG
5. SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP-labeled streptoavidin
6. Enzyme Substrate Solution	Liquid	1 bottle (12 mL)	3,3'5,5'-tetramethyl benzidine (TMB)
7. Stop Solution	Liquid	1 bottle (12 mL)	1M H_2SO_4
8. Buffer Solution	Liquid	1 bottle (25 mL)	Phosphate buffer
9. Wash Solution Concentrate	Liquid	1 bottle (25 mL)	Concentrated saline
10. Adhesive Plate Seal		3 sheets	

MTP[*1] ..... Microtiter plate

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 450 nm
- Washing device for microtiter plate with aspiration system and dispenser
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
- Polypropylene test tubes for preparation of Calibrator Solutions
- Graduated cylinder (500 mL)
- Distilled water or de-ionized water

PRECAUTIONS

10~20 fold of 10 mM phosphate buffer (pH 7.4) may be used to extract tissues. Extracted tissue supernatant solution should be adjusted to a pH of about 7.0~7.4. It is recommended that the extracting solution should be added with enzyme inhibitors, e.g. aprotinin and PMSF, then the tissue extract supernatant be lyophilized and re-dissolved with the buffer soultion included in the kit before running the assay. Extract sample should be kept below -30℃ and avoid repeated freezing and thawing of samples.

Calibrator antigen and labeled antigen solutions should be prepared within one hour of use.

During storage of the concentrated washing solution at 4℃, precipitates may be observed. However, they will dissolve when diluted. Diluted washing solution is stable for 6 months at 4℃.

The kit may be split and used twice. In this case, reconstituted reagents should be stored below -30℃ and the rest of the kit may be stored at 4℃.

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

Perform all determinations in duplicate.

Read the optical absorbance of the reaction solution as soon as possible after addition of the Stop Solution.

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

Satisfactory performance of the test is guaranteed only when reagents are used from a kit with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette the prepared Calibrator Solutions or samples into corresponding wells. Use a new tip for each sample and calibrator to avoid cross-contamination. Use clean test tubes or vessels.

Always run a calibration curve when testing samples.

REAGENT PREPARATION

1. Preparation of Calibrator Solutions: Reconstitute the NOS-1 Calibrator (lyophilized human NOS-1, 32.4 pmol/vial) with 1 mL of Buffer Solution, giving a 32.4 pmol/mL Calibrator Solution after reconstitution. 0.2 mL of the reconstituted Calibrator Solution is diluted with 0.4 mL of Buffer Solution to yield a 10.8 pmol/mL Calibrator Solution. Repeat the serial dilution to make Calibrator Solutions at 3.6, 1.2, 0.4 and 0.133 pmol/mL. Buffer Solution is used as the zero calibrator (0 pmol/mL).
   Note: Calibrator Solution must be prepared within 1 hour of being used.

2. Preparation of Labeled Antigen: Reconstitute Labeled Antigen with 12 mL of Buffer Solution.
   Note: Labeled Antigen must be prepared within 1 hour of being used.

3. Preparation of Wash Solution: Dilute 25 mL of Wash Solution Concentrate to 500 mL with distilled or de-ionized water.

4. Other reagents are ready for use.

STORAGE

Store kit at 4°C.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20 - 30°C) for at least one hour before beginning the test.
2. Add 0.35 mL/well of Wash Solution into each of the wells and then aspirate the plate. Repeat this washing procedure twice, for a total of 3 wash steps.
3. Fill 100 µL of Labeled Antigen solution into the wells first, then add 50 µL of each of the prepared Calibrator Solutions or samples into wells, then add 50 µL of NOS-I Antibody into the wells.
4. Cover the plate with the Plate Seal and incubate at room temperature overnight (16-20 hours).
5. Remove the Plate Seal and aspirate the solution in the wells. Wash the wells three times with approximately 0.35 mL/well of Wash Solution.
6. Pipette 100 µL of SA-HRP Solution into the wells.
7. Cover the plate with a Plate Seal and incubate at room temperature for 1 hour.
8. Remove the Plate Seal, aspirate and wash the wells five times with approximately 0.35 mL/well of Wash Solution.
9. Add 100 µL of Substrate Solution into the wells, cover the plate with a Plate Seal and incubate for 30 minutes at room temperature in the dark for the color reaction.
10. Add 100 µL of Stop Solution into the wells to stop the color reaction.
11. Read the optical absorbance of the wells at 450 nm.

RESULTS

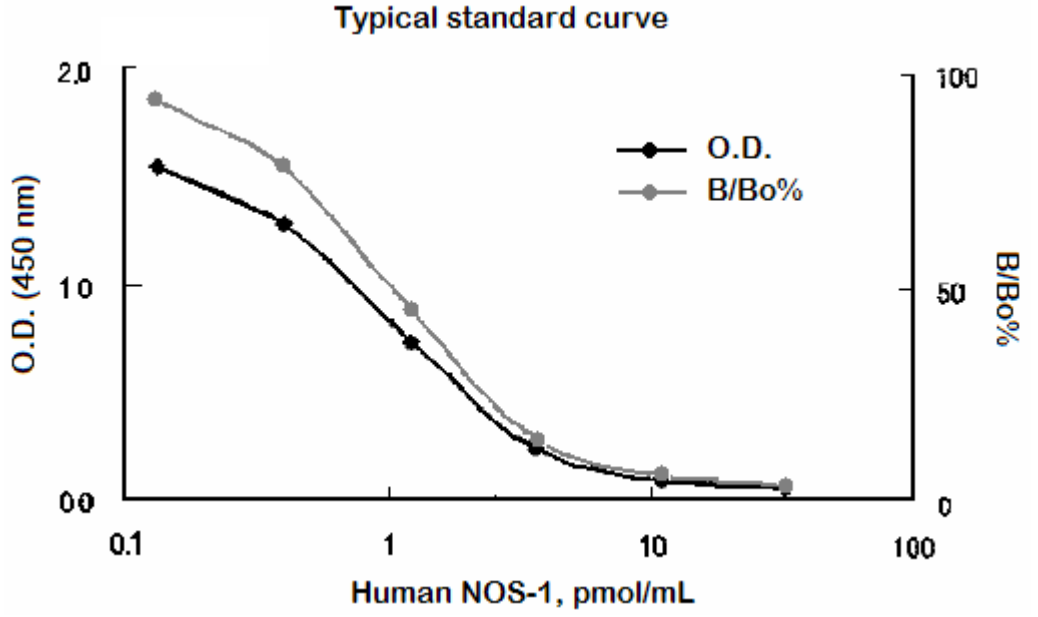
Prepare a calibration curve on semi-logarithmic graph paper by plotting B/Bo% or optical density on the ordinate against concentration of NOS-I on the abscissa. (abscissa: concentration of calibrators; ordinate: B/Bo% or optical density). Calculate B/Bo% for each unknown sample and read values directly from the curve in pmol/mL. If 4-parameter calibration curve fitting software can be used, sample values may easily be 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

PERFORMANCE

Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Analytical Recovery

Sample	Human NOS-1 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 #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 #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	½ Dilution	¼ Dilution
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-1 (998-1024) added to samples, pmol/mL

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

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com