Human eNOS ELISA Kit

Enzyme Immunoassay for the quantification of Human eNOS in Human Serum, plasma and cell culture supernatants.

Catalog number: ARG82055

Package: 96 wells

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INTRODUCTION

Nitric oxide is a reactive free radical which acts as a biologic mediator in several processes, including neurotransmission and antimicrobial and antitumoral activities. Nitric oxide is synthesized from L-arginine by nitric oxide synthases. Variations in this gene are associated with susceptibility to coronary spasm. Multiple transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, May 2009]

Produces nitric oxide (NO) which is implicated in vascular smooth muscle relaxation through a cGMP-mediated signal transduction pathway. NO mediates vascular endothelial growth factor (VEGF)-induced angiogenesis in coronary vessels and promotes blood clotting through the activation of platelets.

Isoform eNOS13C: Lacks eNOS activity, dominant-negative form that may down-regulate eNOS activity by forming heterodimers with isoform 1. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of bound in the initial step. The color development is stopped by the

addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Expiration date: Six months at 4°C and twelve months at-20°C

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	2 X 5 ng/vial	4°C
Standard/Sample diluent	30 ml (Ready to use)	4°C (-80°C after reconstitution)
Antibody conjugate concentrate (100X)	1 vial (120 µl)	4°C
Antibody diluent buffer	12 ml (Ready to use)	4°C
HRP-Streptavidin concentrate (100X)	1 vial (120 µl)	4°C
HRP-Streptavidin diluent buffer	12 ml (Ready to use)	4°C
20X Wash buffer	30 ml	4°C
TMB substrate	12 ml (Ready to use)	4°C (Protect from light)
STOP solution	12 ml (Ready to use)	4°C
Plate sealer	3 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times. Reconstituted standard should be aliquoted and stored at -20°C or -80°C (-80°C is recommended) to avoid repeated freeze-thaw cycles.
- Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8 °C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C for 15 min or until the crystals are completely dissolved.
- To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
- The TMB Color developing agent should be colorless and transparent before using.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Do not let strips dry, as this will inactivate active components in wells.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the 1X HRP-Streptavidin Solution and TMB substrate be pre-warmed in 37°C before use.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect supernatants and assay immediately or aliquot & store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

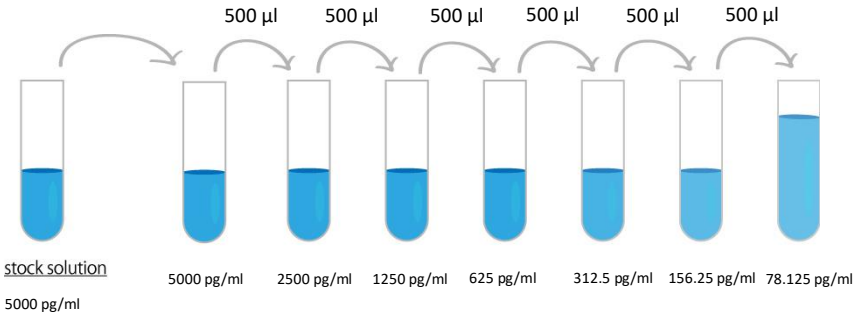
REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X wash buffer with distilled water to yield 1X wash buffer, mix thoroughly. (E.g. 30 ml of 20X Wash buffer + 570 ml of distilled water) The dissolved 1X wash buffer is stable for a week at 2°C to 8°C.
- 1X Antibody conjugate: It is recommended to prepare this reagent immediately prior to use and use it within 1 hours after preparation. Dilute 100X antibody conjugate concentrate into Antibody diluent buffer to yield 1X detection antibody solution, mix thoroughly. (e.g. 10 µl of 100X antibody conjugate concentrate + 990 µl of Antibody diluent buffer)
- 1X HRP-Streptavidin Solution: It is recommended to prepare this reagent immediately prior to use and use it within 1 hours after preparation. Dilute 100X HRP-Streptavidin concentrate solution into HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer, mix thoroughly. (e.g. 10 µl of 100X HRP-Streptavidin concentrate solution + 990 µl of HRP-Streptavidin diluent buffer)
- Sample: If the initial assay found samples contain higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay.

  (It is recommended to do pre-test to determine the suitable dilution factor).

- **Standards**: Standard solution should be prepared within 1 hours prior to the experiment. Reconstitute the standard with **1 ml** of Standard/Sample diluent to yield a stock concentration of **5000 pg/ml.** Allow the stock standard to sit for at least 5 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as according to the suggested concentration below: **5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312.5 pg/ml, 156.25 pg/ml, 78.125 pg/ml**.

  Note: The reconstituted standard solutions are best used within 1 hours. The stock standard solution should be stored at 4°C for up to 12 hours, or aliquot & store at-20°C or -80°C for up to 48 hours. Avoid repeated freeze-thaw cycles



Dilute standard as according to the table below:

Standard	Conc. (pg/ml)	µl of Standard/Sample diluent	µl of standard
S7	5000 pg/ml	0	1000 (5000 pg/ml Stock)
S6	2500 pg/ml	500	500 (S7)
S5	1250 pg/ml	500	500 (S6)
S4	625 pg/ml	500	500 (S5)
S3	312.5 pg/ml	500	500 (S4)
S2	156.25 pg/ml	500	500 (S3)
S1	78.125 pg/ml	500	500 (S2)
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) or 37°C before use. The 1X HRP-Streptavidin Solution and TMB substrate should be prewarm at 37°C few minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of amount in samples. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards, samples and zero controls** (S0, Standard/Sample diluent) into wells. Cover the plate and incubate for **90 minutes** at **room temperature** or overnight at 4°C with gentle shaking.
3. Aspirate each well and wash, repeating the process two times for a total

three washes. Wash by filling each well with 1X Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 1-2 min before remove at each time. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. *DO NOT let the wells completely dry at any time.*

4. Add 100 µl of 1X Antibody conjugate into each well, gently tap the plate to mix well. Cover wells and incubate for 60 minutes at 37°C.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of 1X HRP-Streptavidin solution to each well, gently tap the plate to mix well. Cover wells and incubate for 45 minutes at 37°C.
7. Aspirate each well. Wash as step 3 but wash for a total five washes at this step.
8. Add 100 µl of TMB substrate to each well. Incubate for 30 minutes at 37°C in dark. (Note: The incubation time is for reference only, the optimal incubation time should be determined by end user. And the shades of blue color can be seen in the wells with the four most concentrated standard solutions; the other wells show no obvious color).
9. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
10. Read the OD with a microplate reader at 450nm immediately. It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

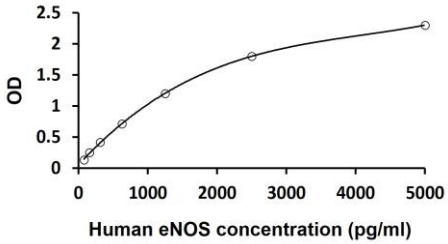
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human eNOS ranged from 78- 5000 pg/ml. The mean MDD was 39 pg/ml.

Specificity

This assay recognizes natural and recombinant Human eNOS. No significant cross-reactivity or interference with the factors below was observed:

There is no detectable cross-reactivity with other relevant proteins.

Intra-assay and Inter-assay precision

The CV values of intra-assay was < 10% and inter-assay was < 10%.