Taiclone®
Quality Guarantee

USER MANUAL



ELISA KIT

ELISA Kit for Endothelial NOS (eNOS) ELISA Kit

Catalog No.tcue152 96 Tests

FOR RESEACH USE ONLY!

Please read completely user manual and storage condition.

Web: www.taiclone.com
Tel: +886-2-2735-9682
Email: order@taiclone.com

ELISA Kit for Endothelial NOS (eNOS) ELISA Kit
Catalog No.tcue12

Available Sizes

Size: 96 Tests

Specifications

Research Area:	Cardiovascular Reseach
Species Reactivity:	Rattus norvegicus (Rat)
Sample Type:	Serum, plasma and Tissue Homogenates, other biological fluids
Sensitivity:	0.65ng/ml
Recovery:	Recovery ranges are from 93-105% .
Detection Range:	1.56~100ng/ml
Assay Time:	3h
Detection Method:	Sandwich ELISA, Double Antibody,Colorimetric; absorbance at 450 nm
Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level eNoS were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level eNOS were tested on 3 different plates, 8 replicates in each plate. Intra-assay coefficient of variation (CV) <8%. Inter-assay CV <10%.

Test Principle

This kit was based on sandwich enzyme-linked immune-sorbent assay technology.
Anti eNOS antibody was pre-coated onto 96-well plates.
And the biotin conjugated anti-eNOS antibody was used as detection antibodies.
The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer.
HRPStreptavidin was added and unbound conjugates were washed away with wash buffer.
TMB substrates were used to visualize HRP enzymatic reaction.
TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. Thedensity of yellow is proportional to the eNOS amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of eNOS can be calculated..

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Materials and Storage.

Store kit components at -20 °C unless specified otherwise.
DO NOT USE past kit expiration date. Some vials contain a small amount of reagents.
Spin tubeson pulse setting prior to opening

Components

Item	Specifications(48T/96T)	Storage
Micro ELISA Plate(Dismountable)	8×6 or 8×12	4°C/-20°C
Lyophilized Standard	1 vial or 2 vial	4°C/-20°C
Sample / Standard dilution buffer	10ml/20ml	4°C
Biotin-detection antibody (Concentrated)	60ul/120ul	4°C
Antibody dilution buffer	5ml/10ml	4°C
HRP-Streptavidin Conjugate(SABC)	60ul/120ul	4°C(shading light)
SABC dilution buffer	5ml/10ml	4°C
TMB substrate	5ml/10ml	4°C(shading light)
Stop solution	5ml/10ml	4°C
Wash buffer (25X)	15ml/30ml	4°C
Plate Sealer	3/5pieces	
Product Description	1 copy	

Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000μL
- Multi-channel pipette calibrated to deliver 50-250μL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm.

Safety Precautions

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Oraliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Tissue homogenates: For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

Cell culture supernate: Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernate and carry out the assay immediately.

Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8°C. Collect the supernatant and carry out the assay immediately.

Sample preparation: Samples should be clear and transparent and be centrifuged to remove suspended solids.

Note: Samples to be used within 5 days may be stored at 4°C,otherwise samples must be stored at -20°C (≤1 month) or -80°C(≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

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Sample Dilution Guideline

End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration falls the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary in practice. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be make in pre-experiment.

High target protein concentration (200-2000ng/ml): Dilution: 1:100. (i.e. Add 1µl of sample into 99 µl of Sample / Standard dilution buffer.)

Medium target protein concentration (20-200ng/ml): Dilution: 1:10.(i.e. Add 10 µl of sample into 90 µl of Sample / Standard dilution buffer.)

Low target protein concentration (0.312-20ng/ml): Dilution: 1:2.(i.e. Add 50 µl of sample into 50 µl of Sample / Standard dilution buffer.)

Very low target protein concentration (≤0.312ng/ml): Unnecessary to dilute, or dilute at 1:2.

Reagent Preparation and Storage

Bring all reagents to room temperature before use.

1, Wash Buffer:

Dilute 30mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2, Standard:

1). 20ng/ml of standard solution: Add 1 ml of Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 min and mix thoroughly.

2). 10ng/ml→0.312ng/ml of standard solutions: Label 6 Eppendorf tubes with 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.312ng/ml, respectively. Aliquot 0.3 ml of the Sample / Standard dilution buffer into each tube. Add 0.3 ml of the above 20ng/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.

Sample Dilution Guideline

End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration falls the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary in practice. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be make in pre-experiment.

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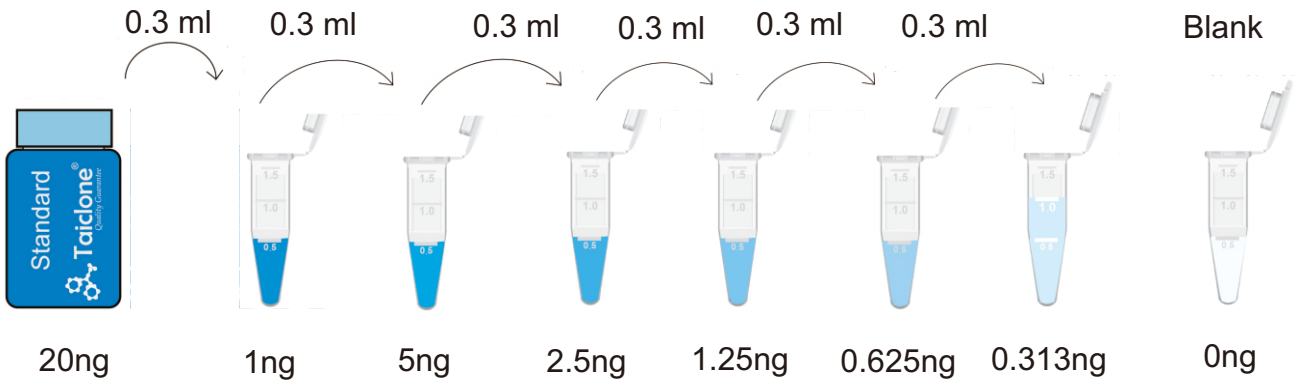

Note: The standard solutions are best used within 2 hours. The standard solution should be at 4°C for up to12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

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3, Preparation of Biotin- detection Antibody working solution
prepare within 1 hour before the experiment.
1) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)
2) Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 µl of Biotin- detection antibody into 99 µl of Antibody dilution buffer.)
4, Preparation of HRP-Streptavidin Conjugate (SABC) working solution:
prepare within 30min before the experiment.
1) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)
2) Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 µl of SABC into 99 µl of SABC dilution buffer.)

This kit is compatible with EDTA-plasma, heparin plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

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Assay Procedure

Before adding to wells, equilibrate TMB substrate for at least 30 min at room temperature (37 °C). When diluting samples and reagents, they must be mixed completely and evenly. It is recommend to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommend to measure each standard and sample in duplicate. Wash plate 2 times before adding standard, sample and control (zero) wells!
2. Aliquot 0.1ml of 20ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.312ng/ml, standard solutions into the standard wells.
3. Add 0.1 ml of Sample / Standard dilution buffer into the control (zero) well.
4. Add 0.1 ml of properly diluted sample (Human serum, plasma, tissue homogenates and other biological fluids.) into test sample wells.
5. Seal the plate with a cover and incubate at 37 °C for 60 min.
6. Remove the cover and discard the plate content, clap the plate on the absorbent filter papers or other absorbent material. Do NOT let the wells completely dry at any time. Do Not Wash Plate!
7. Add 0.1 ml of Biotin- detection antibody working solution into the above wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37°C for 30 min.
9. Remove the cover, and wash plate 3 times with Wash buffer.
10. Add 90ul of TMB substrate solution into each well, cover the plate and incubate at 37°C for 10-20 min.
11. Add 50 µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
12. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The eNOS concentration of the samples can be interpolated from the standard curve. Recommended to use professional software curve expert to 1.3.

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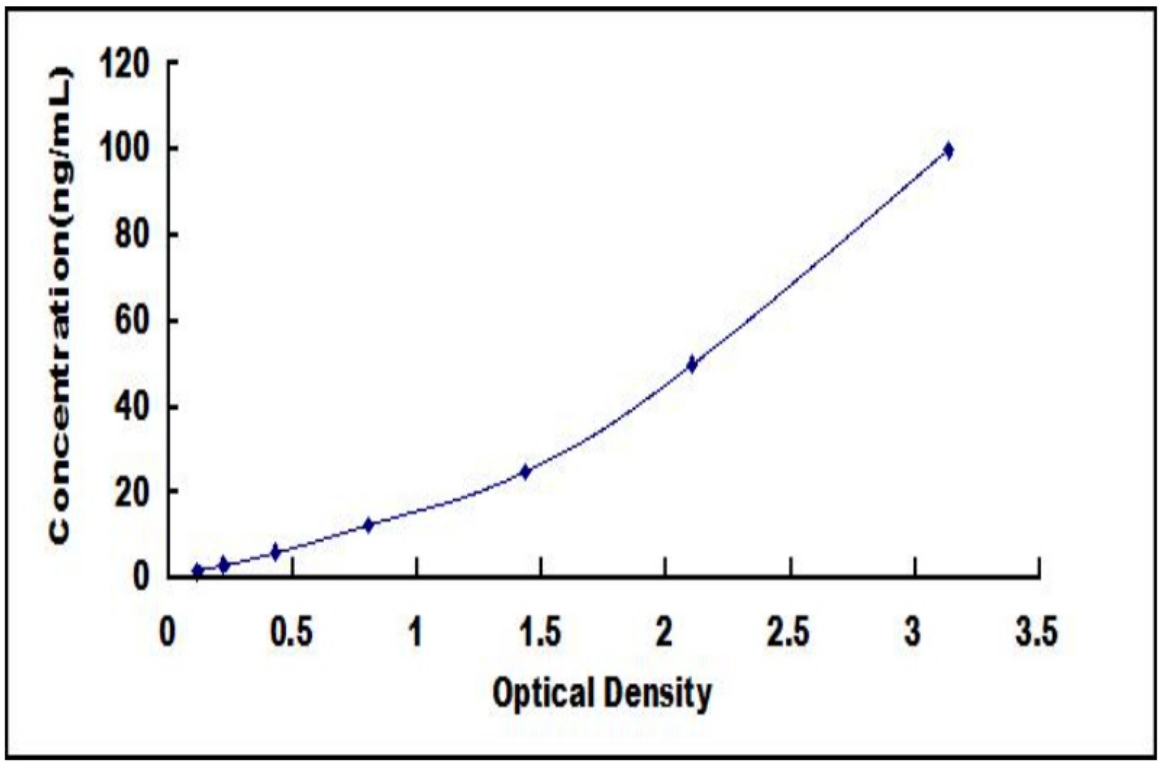
Summary

1. Wash plate 2 times before adding standard, sample and control (zero) wells!
2. Add 100μL standard or sample to each well for 90 minutes at 37°C
3. add 100μL Biotin- detection antibody working solution to each well for 60 minutes at 37°C
4. Aspirate and wash 3 times
5. Add 100μL Reagent B solution to each well. Incubate for 30 minutes at 37°C
6. Aspirate and wash 5 times
7. Add 90μL TMB substrate. Incubate 10 -20 minutes at 37°C
8. Add 50μL Stop Solution. Read at 450nm immediately
9. Calculation of results

Typical Data & Standard Curve

Results of a typical standard run of a eNOS ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	ng/ml	0	0.312	0.625	1.25	2.5	5	10	20
Y	OD450	0.076	0.149	0.222	0.385	0.67	1.097	1.964	2.462



Typical Standard Curve

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Performance Characteristics.

Specificity

This assay has high sensitivity and excellent specificity for detection of eNOS . No significant cross-reactivity or interference between eNOS and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the crossreactivity detection between eNOS and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with certain level of eNOS and the recovery rates were calculated by comparing the measured value to the expected amount of eNOS in samples.

Matrix	Recovery range (%)	Average(%)
serum(n=5)	89-103	96
EDTA plasma(n=5)	88-104	97
heparin plasma(n=5)	93-104	97

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of eNOS and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	85-101%	93-100%	88-104%	93-97%
EDTA plasma(n=5)	85-98%	85-97%	87-101%	84-97%
heparin plasma(n=5)	80-98%	85-99%	81-98%	83-92%

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level eNOS were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level eNOS were tested on 3 different plates, 8 replicates in each plate.
CV (%) = SD/meanX100
Intra-Assay: CV<8%
Inter-Assay: CV<10%

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

Taiclone Biotech Corp.

Tel :+886 2 2735 9682
Fax:+886 2 2735 9807
Email: order@taiclone.com
Website:www.taiclone.com