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APA868Hu01 100µg Active Endothelial NOS (eNOS) Organism Species: *Homo sapiens* (Human) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Arg756~Gln888 Tags: N-terminal His-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose . Original Concentration: 200µg/mL Applications: Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 6.4 Predicted Molecular Mass: 15.8kDa Accurate Molecular Mass: 19kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

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Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[<u>SEQUENCE</u>]

RKMFQ ATIRSVENLQ SSKSTRATIL VRLDTGGQEG LQYQPGDHIG VCPPNRPGLV EALLSRVEDP PAPTEPVAVE QLEKGSPGGP PPGWVRDPRL PPCTLRQALT FFLDITSPPS PQLLRLLSTL AEEPREQQ

[ACTIVITY]

Endothelial Nitric Oxide Synthase (eNOS), also known as Nitric Oxide Synthase 3 (NOS3) or Constitutive NOS (cNOS), is an enzyme that plays a crucial role in maintaining cardiovascular health. It belongs to the nitric oxide synthase (NOS) family of enzymes, which are responsible for catalyzing the production of nitric oxide (NO) from L-arginine. eNOS is primarily expressed in endothelial cells, which line the interior of blood vessels.NOSIP can directly interact with eNOS and induce conformational change or spatial position change of eNOS, thus inhibiting enzyme activity of eNOS. Thus a functional ELISA assay was conducted to detect the interaction of recombinant human eNOS and recombinant human NOSIP. Briefly, eNOS was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µ I were then transferred to NOSIP-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-eNOS pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 °C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 $^\circ$ C. Finally, add 50 μ L stop solution to the wells and read at 450/630nm immediately. The binding activity of recombinant rat eNOS and recombinant human NOSIP was shown in Figure 1, the EC50 for this effect is 0.05ug/mL.

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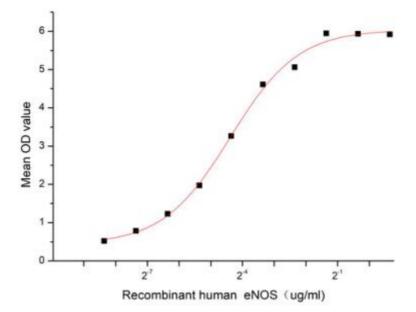


Figure 1. The binding activity of recombinant human eNOS and human NOSIP

[IDENTIFICATION]

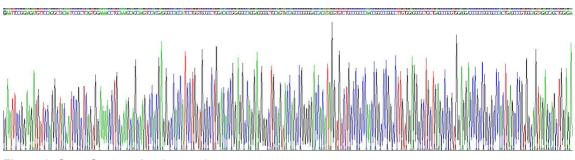


Figure 2. Gene Sequencing (extract)

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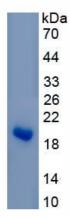


Figure 3. SDS-PAGE

Sample: Active recombinant eNOS, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.