APA628Mu01 100µg Active Nitric Oxide Synthase Trafficker (NOSTRIN) Organism Species: *Mus musculus (Mouse) 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: Thr6~His245 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: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 9.6 Predicted Molecular Mass: 31.5kDa Accurate Molecular Mass: 34kDa 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.

Cloud-Clone Corp.

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.

[SEQUENCE]

TDCSY NKVYKSLKEF AQHGDNFCKQ ITSVLQQRAN LEISYAKGLQ KLAVRLSKAL QSTKKNCLST AWAWASESMK SAADLHQKLG KAIELEAIKP THQVLSMQEK KRKSLDNEVE KTANLVINNW NQQIKAKKKL MMSTKKHEAL FHLVESSKQS LTQKEKQKLL NKLKKSTEKL EKEDESYYQK NMAGYSTRLK WESTLENCYK SMLELEKERI QLLCNNLNQY SQHISLFGQT LTTCH

[ACTIVITY]

Nitric Oxide Synthase Trafficker, abbreviated as NOSTRIN, is a protein that plays a role in the regulation of nitric oxide synthase (NOS) activity. NOSTRIN is involved in the internalization and trafficking of endothelial NOS (eNOS) from the plasma membrane to intracellular compartments, which affects the production of nitric oxide (NO). NO is a signaling molecule that has a variety of physiological functions, including the regulation of blood pressure and vascular tone. By modulating the localization and activity of eNOS, NOSTRIN can influence these processes. NOSTRIN is expressed in various tissues, with particularly high levels in the endothelium, and its expression is regulated by factors such as shear stress and inflammatory cytokines. Alterations in NOSTRIN function have been implicated in several cardiovascular diseases.Caveolin 1 (CAV1) is a protein that is known to interact with NOSTRIN. This interaction is significant in various cellular processes, including the regulation of lipid metabolism and signal transduction pathways. Thus a functional ELISA assay was conducted to detect the interaction of recombinant mouse NOSTRIN and recombinant mouse CAV1. Briefly, NOSTRIN was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µ I were then transferred to CAV1-coated microtiter wells and incubated for 1h at 37 $^\circ\!\!C$. Wells were washed with PBST and incubated for 1h with

Cloud-Clone Corp.

anti-NOSTRIN pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 $^{\circ}$ 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 µL stop solution to the wells and read at 450/630nm immediately. The binding activity of recombinant mouse NOSTRIN and recombinant mouse CAV1 was shown in Figure 1, the EC50 for this effect is 0.10ug/mL.



Figure 1. The binding activity of recombinant mouse NOSTRIN and recombinant mouse

CAV1

[IDENTIFICATION]

Cloud-Clone Corp.

kDa 70
44
33
26
22
18
14
10

Figure 2. SDS-PAGE

Sample: Active recombinant NOSTRIN, Mouse

[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.