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NOS3 siRNA (ovine): sc-270092

BACKGROUND

Nitric oxide (NO) has a broad range of biological activities and has been implicated in signaling pathways in phylogenetically diverse species. Nitric oxide synthases (NOSs), the enzymes responsible for synthesis of NO, contain an N-terminal oxygenase domain and a C-terminal reductase domain. NOS activity requires homodimerization as well as three cosubstrates (L-arginine, NADPH and O₂) and five cofactors or prosthetic groups (FAD, FMN, calmodulin, tetrahydrobiopterin and heme). Several distinct NOS isoforms have been described and been shown to represent the products of three distinct genes. These include two constitutive Ca²⁺/CaM-dependent forms of NOS, including NOS1 (also designated ncNOS) whose activity was first identified in neurons, and NOS3 (also designated ecNOS), first identified in endothelial cells. The inducible form of NOS, NOS2 (also designated iNOS), is Ca²⁺-independent and is expressed in a broad range of cell types.

REFERENCES

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- Marietta, M.A. 1994. Nitric oxide synthase: aspects concerning structure and catalysis. *Cell* 78: 927-930.
- Heiss, L.N., et al. 1994. Epithelial autotoxicity of nitric oxide: role in the respiratory cytopathology of pertussis. *Proc. Natl. Acad. Sci. USA* 91: 267-270.
- Farias-Eisner, R., et al. 1994. Nitric oxide is an important mediator for tumoricidal activity *in vivo*. *Proc. Natl. Acad. Sci. USA* 91: 9407-9411.
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- Bukrinsky, M.E., et al. 1995. Regulation of nitric oxide synthase activity in human immunodeficiency virus type 1 (HIV-1)-infected monocytes: implications for HIV-associated neurological disease. *J. Exp. Med.* 181: 735-745.

CHROMOSOMAL LOCATION

Genetic locus: NOS3 (ovine) mapping to 4.

PRODUCT

NOS3 siRNA (ovine) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NOS3 shRNA Plasmid (ovine): sc-270092-SH and NOS3 shRNA (ovine) Lentiviral Particles: sc-270092-V as alternate gene silencing products.

For independent verification of NOS3 (ovine) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270092A, sc-270092B and sc-270092C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μl of RNase-free water makes a 10 μM solution in a 10 μM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NOS3 siRNA (ovine) is recommended for the inhibition of NOS3 expression in ovine cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μM in 66 μl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

NOS3 (A-9): sc-376751 is recommended as a control antibody for monitoring of gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ovine gene expression knockdown using RT-PCR Primer: NOS3 (ovine)-PR: sc-270092-PR (20 μl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.