SANTA CRUZ BIOTECHNOLOGY, INC.

NOXO1 siRNA (m): sc-75950

BACKGROUND

NADPH oxidase (NOX) proteins include a membrane-bound flavocytochrome containing two subunits (gp91 phox and p22 phox) and the cytosolic proteins p47 phox and p67 phox. NOX activation leads to the formation of a complex that catalyzes the transfer of electrons from NADPH to molecular oxygen, therefore generating reactive oxygen species (ROS). NOXO1 (NADPH oxidase organizer 1), also designated SH3 and PX domain-containing protein 5 and Nox-organizing protein 1, is a 376 amino acid protein that targets NOX to different subcellular compartments and also targets NOX activators to NOX. Interestingly, NOXO1 is required for the synthesis of otoliths, cystalline structures of the inner ear that are involved in the perception of gravity. There are four isoforms of NOXO1 that are produced as a result of alternative splicing events.

REFERENCES

1. Bánfi, B., et al. 2003. Two novel proteins activate superoxide generation by the NADPH oxidase NOX1. J. Biol. Chem. 278: 3510-3513.
2. Geiszt, M., et al. 2003. Proteins homologous to p47phox and p67phox support superoxide production by NAD(P)H oxidase 1 in colon epithelial cells. J. Biol. Chem. 278: 20006-20012.
3. Takeya, R., et al. 2003. Novel human homologues of p47phox and p67phox participate in activation of superoxide-producing NADPH oxidases. J. Biol. Chem. 278: 25234-25246.
4. Cheng, G., et al. 2004. NOXO1, regulation of lipid binding, localization, and activation of Nox1 by the Phox homology (PX) domain. J. Biol. Chem. 279: 4737-4742.
5. Bánfi, B., et al. 2004. NOX3, a superoxide-generating NADPH oxidase of the inner ear. J. Biol. Chem. 279: 46065-46072.
6. Cheng, G., et al. 2005. Alternative mRNA splice forms of NOXO1: differential tissue expression and regulation of Nox1 and Nox3. Gene 356: 118-126.
7. Ueno, N., et al. 2005. The NADPH oxidase Nox3 constitutively produces superoxide in a p22phox-dependent manner: its regulation by oxidase organizers and activators. J. Biol. Chem. 280: 23328-23339.
8. Park, H.S., et al. 2006. Molecular interaction of NADPH oxidase 1 with βPix and Nox Organizer 1. Biochem. Biophys. Res. Commun. 339: 985-990.

CHROMOSOMAL LOCATION

Genetic locus: Noxo1 (mouse) mapping to 17 A3.3.

PRODUCT

NOXO1 siRNA (m) 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 NOXO1 shRNA Plasmid (m): sc-75950-SH and NOXO1 shRNA (m) Lentiviral Particles: sc-75950-V as alternate gene silencing products.

For independent verification of NOXO1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-75950A, sc-75950B and sc-75950C.

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

NOXO1 siRNA (m) is recommended for the inhibition of NOXO1 expression in mouse 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

NOXO1 (F-5): sc-390927 is recommended as a control antibody for monitoring of NOXO1 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 NOXO1 gene expression knockdown using RT-PCR Primer: NOXO1 (m)-PR: sc-75950-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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