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Nox3 shRNA (h) Lentiviral Particles: sc-45484-V

BACKGROUND

Nox3 (GP91-3, NADPH oxidase 3) is a plasma membrane-associated enzyme that catalyzes the production of superoxide by a one-electron reduction of oxygen, using NADPH as the electron donor. Nox3 contains six membrane-spanning regions, conserved flavin and pyridine nucleotide-binding sites, and histidines possibly involved in heme ligation. It functions together with p22phox as an enzyme constitutively producing superoxide. Nox3 expression promotes p22phox transport to the plasma membrane and can be inhibited by mutations in the p22phox binding sites (SH3 domains) of p47phox or Nox1. Nox3 localizes to the vestibular and cochlear sensory epithelia and to spiral ganglions and participates in otoconia formation in inner ears, which is required for perception of balance and gravity.

REFERENCES

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CHROMOSOMAL LOCATION

Genetic locus: NOX3 (human) mapping to 6q25.3.

PRODUCT

Nox3 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Nox3 siRNA (h): sc-45484 and Nox3 shRNA Plasmid (h): sc-45484-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

Nox3 shRNA (h) Lentiviral Particles is recommended for the inhibition of Nox3 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Nox3 (A-12): sc-34699 is recommended as a control antibody for monitoring of Nox3 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Nox3 gene expression knockdown using RT-PCR Primer: Nox3 (h)-PR: sc-45484-PR (20 μ l, 470 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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