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Nox5 shRNA (h) Lentiviral Particles: sc-45486-V

BACKGROUND

The superoxide-generating NADPH oxidase includes a membrane-bound flavocytochrome containing two subunits (gp91-phox and p22-phox) and the cytosolic proteins p47-phox and p67-phox. During activation of the NADPH oxidase, p47-phox and p67-phox migrate to the plasma membrane, where they associate with the flavocytochrome cytochrome b558 to form the active enzyme complex. The p22- and gp91-phox subunits also function as surface O₂ sensors that initiate cellular signaling in response to hypoxic conditions. NADPH oxidase 5 (Nox5) is a homolog of the gp91-phox subunit of the phagocyte NADPH oxidase. Nox5 is expressed in lymphoid organs and testis and is distinguished from the other NADPH oxidases by its unique N-terminus, which contains three canonical EF-handed, Ca²⁺-binding domains. Upon heterologous expression, Nox5 generates superoxide in response to intracellular Ca²⁺ elevations.

REFERENCES

1. Ushio-Fukai, M., et al. 1996. p22-phox is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates Angiotensin II-induced hypertrophy in vascular smooth muscle cells. *J. Biol. Chem.* 271: 23317-23321.
2. Nisimoto, Y., et al. 1999. The p67-(phox) activation domain regulates electron flow from NADPH to flavin in flavocytochrome b(558). *J. Biol. Chem.* 274: 22999-23005.
3. Archer, S.L., et al. 1999. O₂ sensing is preserved in mice lacking the gp91-phox subunit of NADPH oxidase. *Proc. Natl. Acad. Sci. USA* 96: 7944-7949.
4. Geiszt, M., et al. 2000. Identification of renox, an NAD(P)H oxidase in kidney. *Proc. Natl. Acad. Sci. USA* 97: 8010-8014.
5. Cheng, G., et al. 2001. Homologs of gp91-phox: cloning and tissue expression of Nox3, Nox4, and Nox5. *Gene* 269: 131-140.

CHROMOSOMAL LOCATION

Genetic locus: NOX5 (human) mapping to 15q23.

PRODUCT

Nox5 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 µl frozen stock containing 1.0 x 10⁶ 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 Nox5 siRNA (h): sc-45486 and Nox5 shRNA Plasmid (h): sc-45486-SH as alternate gene silencing products.

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.

APPLICATIONS

Nox5 shRNA (h) Lentiviral Particles is recommended for the inhibition of Nox5 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

Nox5 (W-15): sc-34707 is recommended as a control antibody for monitoring of Nox5 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 Nox5 gene expression knockdown using RT-PCR Primer: Nox5 (h)-PR: sc-45486-PR (20 µl, 419 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.

PROTOCOLS

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