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SANTA CRUZ BIOTECHNOLOGY, INC.

Mox1 shRNA (h) Lentiviral Particles: sc-43939-V

BACKGROUND

Mox1 and the glycoprotein gp91-phox are largely related proteins that are essential components of the NADPH oxidase. The superoxide-generating NADPH oxidase is present in phagocytes, neuroepithelial bodies, vascular smooth muscle cells and endothelial cells. It 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 O_2 sensors that initiate cellular signaling in response to hypoxic conditions. Mox1 and gp91 contain identical C-terminal sequence identity, yet they have distinct expression patterns. gp91-phox is expressed in eosinophils, neutrophils, monocytes and B lymphocytes, whereas Mox1 is predominantly detected in the colon, and low expression is also detected in the uterus and prostate. Mox1 is also upregulated in vascular smooth muscle cells in response to PDGF stimulation, which collectively indicates that Mox1 may function analogously to gp91-phox, yet regulate the NADPH superoxide production in non-phagocytic cells.

REFERENCES

1. Henderson, L.M., et al. 1995. The arachidonate-activable, NADPH oxidase-associated H^+ channel. Evidence that gp91-phox functions as an essential part of the channel. J. Biol. Chem. 270: 5909-5916.
2. Ushio-Fukai, M., et al. 1996. p22phox 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.
3. Suh, Y.A., et al. 1999. Cell transformation by the superoxide-generating oxidase Mox1. Nature 401: 79-82.
4. Archer, S.L., et al. 1999. O_2 sensing is preserved in mice lacking the gp91-phox subunit of NADPH oxidase. Proc. Natl. Acad. Sci. USA 96: 7944-7949.

CHROMOSOMAL LOCATION

Genetic locus: NOX1 (human) mapping to Xq22.1.

PRODUCT

Mox1 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 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 Mox1 siRNA (h): sc-43939 and Mox1 shRNA Plasmid (h): sc-43939-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

Mox1 shRNA (h) Lentiviral Particles is recommended for the inhibition of Mox1 expression in human cells.

SUPPORT REAGENTS

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

Mox1 (H-75): sc-25545 is recommended as a control antibody for monitoring of Mox1 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 goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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 goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Mox1 gene expression knockdown using RT-PCR Primer: Mox1 (h)-PR: sc-43939-PR (20 µl, 515 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.

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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