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LOXL3 shRNA (m) Lentiviral Particles: sc-45225-V

BACKGROUND

Lysyl oxidase (LOX) proteins belong to a family of enzymes that oxidize primary amine substrated to reactive aldehydes. In fibrillar collagens and elastin, LOX catalyzes the lysine-derived cross-links of collagen fibrils and insoluble elastic fibers in the extracellular matrix. It can localize both to the nucleus and the cytoplasm. LOX is involved in tumor suppression, cell motility, cellular senescence and developmental regulation. There are four homologs of LOX, lysyl oxidase-like proteins, designated LOX-like (LOXL1-LOXL4) proteins. LOXL3 is an extracellular protein that localizes specifically to sites of elastogenesis. LOXL2 and LOXL3 can interact and cooperate with the SNAIL protein to down-regulate E-cadherin expression. In epithelial cells, overexpression of LOXL2 or LOXL3 may induce an epithelial-mesenchymal transitions process, an important element in tumor progression. LOXL3 is a widely expressed protein with highest levels of expression in placenta, small intestine, testis, heart, ovary and spleen.

REFERENCES

1. Jourdan-Le Saux, C., et al. 1999. The LOXL2 gene encodes a new lysyl oxidase-like protein and is expressed at high levels in reproductive tissues. *J. Biol. Chem.* 274: 12939-12944.
2. Huang, Y. et al. 2001. Cloning and characterization of a human lysyl oxidase-like 3 gene (hLOXL3). *Matrix Biol.* 20: 153-157.
3. Jourdan-Le Saux, C., et al. 2001. Central nervous system, uterus, heart, and leukocyte expression of the LOXL3 gene, encoding a novel lysyl oxidase-like protein. *Genomics* 74: 211-218.
4. Maki, J.M., et al. 2001. Cloning and characterization of a fourth human lysyl oxidase isoenzyme. *Biochem. J.* 355: 381-387.

CHROMOSOMAL LOCATION

Genetic locus: *Loxl3* (mouse) mapping to 6 C3.

PRODUCT

LOXL3 shRNA (m) 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 LOXL3 siRNA (m): sc-45225 and LOXL3 shRNA Plasmid (m): sc-45225-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.

PROTOCOLS

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

APPLICATIONS

LOXL3 shRNA (m) Lentiviral Particles is recommended for the inhibition of LOXL3 expression in mouse 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

LOXL3 (H-70): sc-68939 is recommended as a control antibody for monitoring of LOXL3 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 LOXL3 gene expression knockdown using RT-PCR Primer: LOXL3 (m)-PR: sc-45225-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.