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LOXL2 siRNA (m): sc-45223

BACKGROUND

Lysyl oxidase (LOX) proteins belong to a family of enzymes that oxidase 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 supression, cell motility, cellular senescence and developmental regulation. There are four homologs of LOX, lysyl oxidase-like proteins designated LOX-like (LOXL1-LOXL4) proteins. LOXL2 is an extracellular protein that localizes specifically to sites of elastogenesis. It serves as a cross-linking enzyme, controlling the deposition of elastin and interacts with Fibulin-5. LOXL2 and LOXL3 can interact and cooperate with the snail protein to downregulate E-cadherin expression. In epithelial cells, overexpression of LOXL2 or LOXL3 may induce an epithelial-mesenchymal transitions process, an important element in tumor progression. Knockdown of the LOXL2 protein significantly decreases tumor growth.

REFERENCES

1. Jourdan-Le Saux, C., et al. 1999. The LOXL2 gene encodes a new LOXL protein and is expressed at high levels in reproductive tissues. *J. Biol. Chem.* 274: 12939-12944.
2. Csiszar, K., et al. 2001. LOX: a novel multifunctional amine oxidase family. *Prog. Nucleic Acid Res. Mol. Biol.* 70: 1-32.
3. Kirschmann, DA., et al. 2002. A molecular role for LOX in breast cancer invasion. *Cancer Res.* 62: 4478-4483.
4. Molnar, J., et al. 2003. Structural and functional diversity of LOX and the LOX-like proteins. *Biochim. Biophys. Acta* 1647: 220-224.
5. Peinado, H., et al. 2005. A molecular role for LOXL2 enzyme in snail regulation and tumor progression. *EMBO J.* 24: 3446-3458.
6. Vadasz, Z., et al. 2005. Abnormal deposition of Collagen around hepatocytes in Wilson's disease is associated with hepatocyte specific expression of LOX and LOXL2. *J. Hepatol.* 43: 499-507.

CHROMOSOMAL LOCATION

Genetic locus: Loxl2 (mouse) mapping to 14 D2.

PRODUCT

LOXL2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see LOXL2 shRNA Plasmid (m): sc-45223-SH and LOXL2 shRNA (m) Lentiviral Particles: sc-45223-V as alternate gene silencing products.

For independent verification of LOXL2 (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-45223A, sc-45223B and sc-45223C.

PROTOCOLS

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

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

LOXL2 siRNA (m) is recommended for the inhibition of LOXL2 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 μ M in 66 μ l. 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

LOXL2 (3C5): sc-293427 is recommended as a control antibody for monitoring of LOXL2 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 LOXL2 gene expression knockdown using RT-PCR Primer: LOXL2 (m)-PR: sc-45223-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.