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15-LO2 shRNA (m) Lentiviral Particles: sc-45627-V

BACKGROUND

Lipoxygenases are a family of enzymes which dioxygenate unsaturated fatty acids, thus initiating lipoperoxidation of membranes, the synthesis of signalling molecules as well as inducing structural and metabolic changes in the cell. The Lox enzymes in mammals, 12-LO and 15-LO, are classified with respect to their positional specificity of the deoxygenation of their most common substrate, arachidonic acid. The metabolism of arachidonic acid leads to the generation of biologically active metabolites that have been implicated in cell growth and proliferation, as well as survival and apoptosis. 15-Lipoxygenase acts in physiological membrane remodeling and the pathogenesis of atherosclerosis, inflammation and carcinogenesis. It is highly regulated and expressed in a tissue- and cell-type-specific fashion. IL-4 and IL-13 play important roles in transactivating the 15-LO gene. Overexpression of 15-LO type 1 in prostate cancer contributes to the cancer progression by regulating IGF-1R expression and activation. 15-lipoxygenase, type II (15-LO2) is important for the conversion of arachidonic acid to 15S-hydroperoxyeicosate-traenoic acid. It is a cytoplasmic protein expressed primarily in cornea, lung, hair and prostate.

REFERENCES

1. Fletcher-Cieutat, M., et al. 1985. Aspirin enhances the sensitivity of human platelet 12-lipoxygenase to inhibition by 15-HETE, an endogenous regulator. *Prostaglandins Leukot. Med.* 18: 255-259.
2. Kilty, I., et al. 1999. Differential characteristics of human 15-LO isozymes and a novel splice variant of 15S-LO. *Eur. J. Biochem.* 266: 83-93.
3. Tang, S., et al. 2002. Evidence that arachidonate 15-LO2 is a negative cell cycle regulator in normal prostate epithelial cells. *J. Biol. Chem.* 277: 16189-16201
4. Lutteke, T., et al. 2003. LOX-DB—database on lipoxygenases. *Bioinformatics* 19: 2482-2483.
5. Pidgeon, G.P., et al. 2003. Overexpression of platelet-type 12-LO promotes tumor cell survival by enhancing $\alpha V/\beta 3$ and $\alpha V/\beta 5$ integrin expression. *Cancer Res.* 63: 4258-4267.

CHROMOSOMAL LOCATION

Genetic locus: Alox8 (mouse) mapping to 11 B4.

PRODUCT

15-LO2 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 15-LO2 siRNA (m): sc-45627 and 15-LO2 shRNA Plasmid (m): sc-45627-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

15-LO2 shRNA (m) Lentiviral Particles is recommended for the inhibition of 15-LO2 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

15-LO2 (D-9): sc-271290 is recommended as a control antibody for monitoring of 15-LO2 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor 15-LO2 gene expression knockdown using RT-PCR Primer: 15-LO2 (m)-PR: sc-45627-PR (20 μ l). 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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