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- Trockeneiszuschlag
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12-LO shRNA (m) Lentiviral Particles: sc-45985-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. The 12-LO pathway is a regulator of cell survival and apoptosis and affects the expression and localization of the Integrin $\alpha V/\beta 5$ and Actin microfilaments in rat Walker 256 carcinosarcoma cells. Platelet-type 12-LO regulates the growth and survival of a number of cancer cells. Human platelets metabolize arachidonic acid via 12-lipoxygenase to 12-hydroxyeicosatetraenoic acid.

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. Pidgeon, G.P., et al. 2003. Overexpression of platelet-type 12-lipoxygenase promotes tumor cell survival by enhancing $\alpha V/\beta 3$ and Integrin $\alpha V/\beta 5$ expression. *Cancer Res.* 63: 4258-4267.
3. Rásó, E., et al. 2004. Molecular identification, localization and function of platelet-type 12-lipoxygenase in human melanoma progression, under experimental and clinical conditions. *Melanoma Res.* 14: 245-250.

CHROMOSOMAL LOCATION

Genetic locus: Alox12 (mouse) mapping to 11 B3.

PRODUCT

12-LO 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 12-LO siRNA (m): sc-45985 and 12-LO shRNA Plasmid (m): sc-45985-SH as alternate gene silencing products.

APPLICATIONS

12-LO shRNA (m) Lentiviral Particles is recommended for the inhibition of 12-LO expression in mouse 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.

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

12-LO (H-100): sc-32939 is recommended as a control antibody for monitoring of 12-LO 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 12-LO gene expression knockdown using RT-PCR Primer: 12-LO (m)-PR: sc-45985-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.

PROTOCOLS

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