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15-LO siRNA (h): sc-105003

BACKGROUND

Lipoxygenases are a family of enzymes which dioxygenate unsaturated fatty acids, thus initiating lipoperoxidation of membranes and synthesis of signaling molecules, as well as inducing structural and metabolic changes in the cell. The Lox enzymes in mammals include 12-LO and 15-LO, which 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 (15-LO) 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.

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 $\alpha(v)\beta(5)$ integrin expression. *Cancer Res.* 63: 4258-4267.
3. Liu C., et al. 2004. Transcriptional regulation of 15-lipoxygenase expression by promoter methylation. *Exp. Cell Res.* 297:61-67.
4. Kelavkar U.P., et al. 2004. 15-lipoxygenase-1 expression upregulates and activates Insulin-like growth factor-1 receptor in prostate cancer cells. *Neoplasia* 6:41-52.
5. Raso 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: ALOX15 (human) mapping to 17p13.2.

PRODUCT

15-LO siRNA (h) 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 15-LO shRNA Plasmid (h): sc-105003-SH and 15-LO shRNA (h) Lentiviral Particles: sc-105003-V as alternate gene silencing products.

For independent verification of 15-LO (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-105003A, sc-105003B and sc-105003C.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

15-LO siRNA (h) is recommended for the inhibition of 15-LO expression in human 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

15-LO (B-7): sc-133085 is recommended as a control antibody for monitoring of 15-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-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-LO gene expression knockdown using RT-PCR Primer: 15-LO (h)-PR: sc-105003-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.