SANTA CRUZ BIOTECHNOLOGY, INC.

15-LO2 siRNA (h): sc-45626

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

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-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. 15-LO, type II (15-LO2) is important for the conversion of arachidonic acid to 15S-hydroperoxyeicosatetraenoic 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.
6. Liu, C., et al. 2004. Transcriptional regulation of 15-LO expression by promoter methylation. Exp. Cell Res. 297: 61-67.

CHROMOSOMAL LOCATION

Genetic locus: ALOX15B (human) mapping to 17p13.1.

PRODUCT

15-LO2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see 15-LO2 shRNA Plasmid (h): sc-45626-SH and 15-LO2 shRNA (h) Lentiviral Particles: sc-45626-V as alternate gene silencing products.

For independent verification of 15-LO2 (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-45626A, sc-45626B and sc-45626C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

15-LO2 siRNA (h) is recommended for the inhibition of 15-LO2 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 µM in 66 µl. 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-LO2 (F-10): sc-376795 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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 15-LO2 gene expression knockdown using RT-PCR Primer: 15-LO2 (h)-PR: sc-45626-PR (20 µl, 428 bp). 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.

PROTOCOLS

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