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12-LO siRNA (h): sc-45984

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 α_V/β_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., Vanderhoek, J.Y., Bryant, R.W. and Bailey, J.M. 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., Tang, K., Cai, Y.L., Piasentin, E. and Honn, K.V. 2003. Over-expression of platelet-type 12-lipoxygenase promotes tumor cell survival by enhancing α_V/β_3 and Integrin α_V/β_5 expression. *Cancer Res.* 63: 4258-4267.
3. Rásó, E., Döme, B., Somlai, B., Zacharek, A., Hagmann, W., Honn, K.V. and Tímár, J. 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 (human) mapping to 17p13.1.

PRODUCT

12-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 12-LO shRNA Plasmid (h): sc-45984-SH and 12-LO shRNA (h) Lentiviral Particles: sc-45984-V as alternate gene silencing products.

For independent verification of 12-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-45984A, sc-45984B and sc-45984C.

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.

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

12-LO shRNA Plasmid (h) is recommended for the inhibition of 12-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

12-LO (C-5): sc-365194 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 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 12-LO gene expression knockdown using RT-PCR Primer: 12-LO (h)-PR: sc-45984-PR (20 μ l, 425 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.