SANTA CRUZ BIOTECHNOLOGY, INC.

Leukocyte-type 12-LO siRNA (m): sc-146714

BACKGROUND

Lipoxygenases are a family of enzymes that 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. Leukocyte-type 12-LO is a 663 amino acid lipoxygenase that is found in leukocytes, aorta, kidney, small intestine and the pineal and pituitary glands. Leukocyte-type 12-LO binds one iron ion per subunit and contains one lipoxygenase domain and one PLAT domain.

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. Freire-Moar, J., et al. 1995. Cloning and characterization of a murine macrophage lipoxygenase. Biochim. Biophys. Acta 1254: 112-116.
3. Pidgeon, G.P., et al. 2003. Overexpression of platelet-type 12-lipoxygenase promotes tumor cell survival by enhancing $\alpha_v b_3$ and $\alpha_v b_5$ Integrin expression. Cancer Res. 63: 4258-4267.
4. Liu, C., et al. 2004. Transcriptional regulation of 15-lipoxygenase expression by promoter methylation. Exp. Cell Res. 297: 61-67.
5. 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.
6. 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 (mouse) mapping to 11 B3.

PRODUCT

Leukocyte-type 12-LO siRNA (m) 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 Leukocyte-type 12-LO shRNA Plasmid (m): sc-146714-SH and Leukocyte-type 12-LO shRNA (m) Lentiviral Particles: sc-146714-V as alternate gene silencing products.

For independent verification of Leukocyte-type 12-LO (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-146714A, sc-146714B and sc-146714C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

Leukocyte-type 12-LO siRNA (m) is recommended for the inhibition of Leukocyte-type 12-LO expression in mouse 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

Leukocyte-type 12-LO (I-17): sc-27364 is recommended as a control antibody for monitoring of Leukocyte-type 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Leukocyte-type 12-LO gene expression knockdown using RT-PCR Primer: Leukocyte-type 12-LO (m)-PR: sc-146714-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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