SANTA CRUZ BIOTECHNOLOGY, INC.

5-LO siRNA (m): sc-29597

BACKGROUND

5-lipoxygenase (5-LO) is expressed primarily in polymorphonuclear leukocytes, macrophages, and mast cells. 5-LO performs the first two catalytic reactions in the biosynthesis of leukotrienes, lipid metabolites that induce contractions of airway smooth muscle and increase vascular permeability during anaphylaxis. The cellular localization of 5-LO varies between cell types. In activated blood polymorphonuclear leukocytes 5-LO undergoes calcium dependent translocation from the cytosol to the nuclear envelope. In alveolar macrophages, the majority of 5-LO is localized in the nucleus and, upon activation of these cells, intranuclear 5-LO binds to the nuclear membrane. This intracellular shuttling of 5-LO is dependent on the association with various signaling molecules, phosphorylation and the presence of a distinct nuclear localization signal, which is encoded at the amino terminus of 5-LO.

REFERENCES

1. Matsumoto, T., et al. 1988. Molecular cloning and amino acid sequence of human 5-lipoxygenase. Proc. Natl. Acad. Sci. USA 85: 26-30.
2. Winkler, J.D., et al. 1993. Influence of arachidonic acid on indices of phospholipase A_2 activity in the human neutrophil. Biochem. J. 291: 825-831.
3. Woods, J.W., et al. 1995. 5-lipoxygenase is located in the euchromatin of the nucleus in resting human alveolar macrophages and translocates to the nuclear envelope upon cell activation. J. Clin. Invest. 95: 2035-2046.
4. Pouliot, M., et al. 1996. Colocalization of cytosolic phospholipase A_2, 5-lipoxygenase, and 5-lipoxygenase activating protein at the nuclear membrane of A23187-stimulated human neutrophils. Eur. J. Biochem. 238: 250-258.

CHROMOSOMAL LOCATION

Genetic locus: Alox5 (mouse) mapping to 6 E3.

PRODUCT

5-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 5-LO shRNA Plasmid (m): sc-29597-SH and 5-LO shRNA (m) Lentiviral Particles: sc-29597-V as alternate gene silencing products.

For independent verification of 5-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-29597A, sc-29597B and sc-29597C.

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

5-LO siRNA (m) is recommended for the inhibition of 5-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

5-LO (33): sc-136195 is recommended as a control antibody for monitoring of 5-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-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 5-LO gene expression knockdown using RT-PCR Primer: 5-LO (m)-PR: sc-29597-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kwak, H.J., et al. 2017. 5-LO inhibition ameliorates palmitic acid-induced ER stress, oxidative stress and Insulin resistance via AMPK activation in murine myotubes. Sci. Rep. 7: 5025.
2. Vagnozzi, A.N., et al. 2017. The direct role of 5-lipoxygenase on Tau pathology, synaptic integrity and cognition in a mouse model of tauopathy. Transl. Psychiatry 7: 1288.
3. Tapper, E.B., et al. 2017. Suboptimal implementation of evidence-based therapy for acute variceal hemorrhage: a systematic review of observational studies. Clin. Gastroenterol. Hepatol. 15: 1373-1381.

RESEARCH USE

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