SANTA CRUZ BIOTECHNOLOGY, INC.

group V PLA_2 siRNA (h): sc-44023

BACKGROUND

Phospholipase A_2s (PLA_2s) constitute a family of esterases that hydrolyze the sn-2-acyl ester bond in glycerophospholipid molecules. These enzymes are generally calcium-dependent and have been found both intra- and extracellularly. By hydrolyzing the sn-2 bond in glycerophospholipids, PLA_2s release fatty acids. One such fatty acid, arachidonic acid, generates the substrates for the initiation of the arachidonic acid cascade that produces various eicosanoids (i.e., prostaglandins, leukotrienes and thromboxanes), many of which are potent mediators of inflammation. PLA_2s include both the relatively low molecular weight group I, group II and group V enzymes and the form known as cytoplasmic PLA_2 ($cPLA_2$). $cPLA_2$ is present in macrophages, and hydrolyzes the sn-2 fatty acyl ester bond of phospholipids to produce a free fatty acid and a lysophospholid.

REFERENCES

1. Heinrikson, R.L., et al. 1977. Amino acid sequence of phospholipase A_2-α from the venom of *Crotalus adamanteus*. A new classification of phospholipases A_2 based upon structural determinants. J. Biol. Chem. 252: 4913-4921.
2. Dennis, E.A. 1990. Phospholipase A_2: role and function in inflammation. Adv. Exp. Med. Biol. 275: 1-25.
3. Heinrikson, R.L. and Kezdy, F. 1990. A novel bifunctional mechanism of surface recognition by phospholipase A_2. Adv. Exp. Med. Biol. 279: 37-47.
4. Clark, J.D., et al. 1990. Purification of a 110-kilodalton cytosolic phospholipase A_2 from the human monocytic cell line U937. Proc. Natl. Acad. Sci. USA 87: 7708-7712.
5. Sharp, J.D., et al. 1991. Molecular cloning and expression of human Ca^{2+}-sensitive cytosolic phospholipase A_2. J. Biol. Chem. 266: 14850-14853.
6. Mukherjee, A.B., et al. 1992. Regulation of extracellular phospholipase A_2 activity: implications for inflammatory diseases. DNA Cell Biol. 11: 233-243.
7. Wooton-Kee, C.R., et al. 2004. Group V $sPLA_2$ hydrolysis of low-density lipoprotein results in spontaneous particle aggregation and promotes macrophage foam cell formation. Arterioscler. Thromb. Vasc. Biol. 24: 762-767.

CHROMOSOMAL LOCATION

Genetic locus: PLA2G5 (human) mapping to 1p36.13.

PRODUCT

group V PLA_2 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 group V PLA_2 shRNA Plasmid (h): sc-44023-SH and group V PLA_2 shRNA (h) Lentiviral Particles: sc-44023-V as alternate gene silencing products.

For independent verification of group V PLA_2 (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-44023A, sc-44023B and sc-44023C.

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

group V PLA_2 siRNA (h) is recommended for the inhibition of group V PLA_2 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

group V PLA_2 (C-4): sc-393606 is recommended as a control antibody for monitoring of group V PLA_2 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 group V PLA_2 gene expression knockdown using RT-PCR Primer: group V PLA_2 (h)-PR: sc-44023-PR (20 µl). 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.