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SANTA CRUZ BIOTECHNOLOGY, INC.

group VI iPLA$_2$ siRNA (h): sc-43819

BACKGROUND

Phospholipases catalyze the release of fatty acids from phospholipids. One member of the phospholipase family, iPLA$_2$, is detected as a membrane-bound protein with multiple smaller isoforms, which result from alternative splicing. Two isoforms, Ankyrin-iPLA$_2$-1 and -2, lack the catalytic domain and are thought to be involved in the negative regulation of iPLA$_2$ activity. The SH-iPLA$_2$ isoform is cytoplasmically localized. The human gene encoding iPLA$_2$ maps to chromosome 22q13.1. Another phopholipase, sPLA$_2$, belongs to a family of secretory phospholipases A$_2$, which represent an expanding family of related enzymes. sPLA$_2$ has both membrane bound and secreted forms that are encoded by a single gene. sPLA$_2$ is involved in the regulation of phospholipid metabolism in biomembranes and in eicosanoid biosynthesis.

REFERENCES

1. Scott, D.L., et al. 1991. Structures of free and inhibited human secretory phospholipase A$_2$ from inflammatory exudate. Science 254: 1007-1010.
2. Lehninger, A., et al. 1993. Principles of biochemistry second edition. Worth Publishers.
3. Cupillard, L., et al. 1997. Cloning, chromosomal mapping, and expression of a novel human secretory phospholipase A$_2$. J. Biol. Chem. 272: 15745-15752.
4. Kitadokoro, K., et al. 1998. Crystal structure of human secretory phospholipase A$_2$-IIA complex with the potent indolizine inhibitor 120-1032. J. Biochem. 123: 619-623.
5. Ma, Z., et al. 1999. Human pancreatic islets express mRNA species encoding two distinct catalytically active isoforms of group VI phospholipase A$_2$ (iPLA$_2$) that arise from an exon-skipping mechanism of alternative splicing of the transcript from the iPLA$_2$ gene on chromosome 22q13.1. J. Biol. Chem. 274: 9607-9616.
6. Larsson-Forsell, P.K., et al. 1999. The human calcium-independent phospholipase A$_2$ gene multiple enzymes with distinct properties from a single gene. Eur. J. Biochem. 262: 575-585.

CHROMOSOMAL LOCATION

Genetic locus: PLA2G6 (human) mapping to 22q13.1.

PRODUCT

group VI iPLA$_2$ 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 VI iPLA$_2$ shRNA Plasmid (h): sc-43819-SH and group VI iPLA$_2$ shRNA (h) Lentiviral Particles: sc-43819-V as alternate gene silencing products.

For independent verification of group VI iPLA$_2$ (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-43819A, sc-43819B and sc-43819C.

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 VI iPLA$_2$ siRNA (h) is recommended for the inhibition of group VI iPLA$_2$ 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 VI iPLA$_2$ (D-4): sc-376563 is recommended as a control antibody for monitoring of group VI iPLA$_2$ 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 VI iPLA$_2$ gene expression knockdown using RT-PCR Primer: group VI iPLA$_2$ (h)-PR: sc-43819-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.