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group X sPLA₂ shRNA (h) Lentiviral Particles: sc-43821-V

BACKGROUND

Phospholipases catalyze the release of fatty acids from phospho-lipids. One member of the phospholipase family, iPLA₂, is detected as a membrane-bound protein with multiple smaller isoforms, which result from alternative splicing. Another phospholipase, sPLA₂, belongs to a family of secretory phospholipases A₂, which represent an expanding family of related enzymes. sPLA₂ has both membrane bound and secreted forms that are encoded by a single gene which maps to human chromosome 1p35. sPLA₂ is involved in the regulation of phospholipid metabolism in biomembranes and in eicosanoid biosynthesis. Group X sPLA₂ mRNA is found in various tissues including the lung, thymus, and spleen, and immunohistochemical analysis reveals its expression in splenic macrophages. Group X sPLA₂ is an actively secreted enzyme that maps to human chromosome 16p13.12.

REFERENCES

1. Scott, D.L., et al. 1991. Structures of free and inhibited human secretory phospholipase A₂ 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 A₂. *J. Biol. Chem.* 272: 15745-15752.
4. Kitadokoro, K., et al. 1998. Crystal structure of human secretory phospholipase A₂-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 A₂ (iPLA₂) that arise from an exon-skipping mechanism of alternative splicing of the transcript from the iPLA₂ gene on chromosome 22q13.1. *J. Biol. Chem.* 274: 9607-9616.

CHROMOSOMAL LOCATION

Genetic locus: PLA2G10 (human) mapping to 16p13.12.

PRODUCT

group X sPLA₂ shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see group X sPLA₂ siRNA (h): sc-43821 and group X sPLA₂ shRNA Plasmid (h): sc-43821-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

group X sPLA₂ shRNA (h) Lentiviral Particles is recommended for the inhibition of group X sPLA₂ expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

group X sPLA₂ (L-13): sc-22966 is recommended as a control antibody for monitoring of group X sPLA₂ 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 group X sPLA₂ gene expression knockdown using RT-PCR Primer: group X sPLA₂ (h)-PR: sc-43821-PR (20 µl, 289 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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