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group VI iPLA₂ siRNA (r): sc-270117

BACKGROUND

Phospholipases catalyze the release of fatty acids from phospholipids. One member of the phospholipase family, iPLA₂, is detected as a membrane-bound protein with multiple smaller isoforms, which result from alternative splicing. Two isoforms, Ankyrin-iPLA₂-1 and -2, lack the catalytic domain and are thought to be involved in the negative regulation of iPLA₂ activity. The SH-iPLA₂ isoform is cytoplasmically localized. 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. sPLA₂ 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 A₂ from inflammatory exudate. *Science* 254: 1007-1010.
2. Cupillard, L., et al. 1997. Cloning, chromosomal mapping, and expression of a novel human secretory phospholipase A₂. *J. Biol. Chem.* 272: 15745-15752.
3. 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.
4. 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: Pla2g6 (rat) mapping to 7q34.

PRODUCT

group VI iPLA₂ siRNA (r) 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 iPLA₂ shRNA Plasmid (r): sc-270117-SH and group VI iPLA₂ shRNA (r) Lentiviral Particles: sc-270117-V as alternate gene silencing products.

For independent verification of group VI iPLA₂ (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270117A, sc-270117B and sc-270117C.

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.

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 iPLA₂ siRNA (r) is recommended for the inhibition of group VI iPLA₂ expression in rat 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 iPLA₂ (D-4): sc-376563 is recommended as a control antibody for monitoring of group VI iPLA₂ 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 iPLA₂ gene expression knockdown using RT-PCR Primer: group VI iPLA₂ (r)-PR: sc-270117-PR (20 μl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.