

cPLA₂ siRNA (m): sc-35098

BACKGROUND

Phospholipase A₂s (PLA₂s) 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, PLA₂s 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. PLA₂s include both the relatively low molecular weight type I and type II enzymes and the form known as cytoplasmic PLA₂ (cPLA₂). cPLA₂ is present in the cytosol of various cells and tissues including platelets, macrophages and monoblasts; and preferentially hydrolyzes the Sn-2 position of phospholipid molecules, releasing free arachidonate.

REFERENCES

1. Henrikson, R.L., et al. 1977. Amino acid sequence of phospholipase A₂-α from the venom of *Crotalus adamanteus*. J. Biol. Chem. 252: 4913-4921.
2. Leslie, C.C., et al. 1988. Properties and purification of an arachidonyl hydrolyzing phospholipase A₂ from a macrophage cell line, RAW 264.7. Biochem. Biophys. Acta 963: 476-492.
3. Diez, E., et al. 1990. Purification of a phospholipase A₂ from human monocytic leukaemic U937 cells: calcium-dependent activation and membrane association. J. Biol. Chem. 265: 14654-14661.
4. Clark, J.D., et al. 1990. Purification of a 110 kDa cytosolic phospholipase A₂ from the human monocytic cell U937. Proc. Natl. Acad. Sci. USA 87: 7708-7712.
5. Dennis, E.A. 1990. Phospholipase A₂: role and function in inflammation. Adv. Exp. Med. Biol. 275: 1-25.

CHROMOSOMAL LOCATION

Genetic locus: Pla2g4a (mouse) mapping to 1 G1.

PRODUCT

cPLA₂ siRNA (m) is a target-specific 19-25 nt siRNA 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 cPLA₂ shRNA Plasmid (m): sc-35098-SH and cPLA₂ shRNA (m) Lentiviral Particles: sc-35098-V as alternate gene silencing 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.

RAPPLICATIONS

cPLA₂ siRNA (m) is recommended for the inhibition of cPLA₂ 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

cPLA₂ (4-4B-3C): sc-454 is recommended as a control antibody for monitoring of cPLA₂ 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 cPLA₂ gene expression knockdown using RT-PCR Primer: cPLA₂ (m)-PR: sc-35098-PR (20 μl, 596 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Qi, H.Y., et al. 2011. A cytosolic phospholipase A₂-initiated lipid mediator pathway induces autophagy in macrophages. J. Immunol. 187: 5286-5292.
2. Kim, H.K., et al. 2016. Glutamine prevents late-phase anaphylaxis via MAPK phosphatase 1-dependent cytosolic phospholipase A₂ deactivation. Int. Arch. Allergy Immunol. 171: 61-70.
3. Zhai, L., et al. 2020. Berberine suppresses colonic inflammation in dextran sulfate sodium-induced murine colitis through inhibition of cytosolic phospholipase A₂ activity. Front. Pharmacol. 11: 576496.
4. Zhan, Y., et al. 2021. PLA2G4A promotes right-sided colorectal cancer progression by inducing CD39⁺γδ Tregs polarization. JCI Insight. E-published.

RESEARCH USE

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