SANTA CRUZ BIOTECHNOLOGY, INC.

LPAAT-θ siRNA (h): sc-62565

BACKGROUND

Phosphatidic acid and lysophosphatidic acid are phospholipids involved in lipid biosynthesis and signal transduction. LPAAT-θ (lysophosphatidic acid acyltransferase θ) catalyzes the synthesis of phosphatidic acid from lysophosphatidic acid. LPAAT-θ is a membrane-bound protein belonging to the LPAAT family. Members of the LPAAT family have a well-known role in lipid biosynthesis, and they may also play a role in tumor progression. LPAAT-θ localizes to the endoplasmic reticulum and is expressed in numerous tissue types. Low expression levels are detected in brain, kidney, liver, pancreas, placenta, prostate and thymus. The overexpression of LPAAT-θ can induce FRAP-dependent p70 S6 kinase phosphorylation on Thr389 and 4E-BP1 phosphorylation on Ser65.

REFERENCES

1. West, J., et al. 1997. Cloning and expression of two human lysophosphatidic acid acyltransferase cDNAs that enhance cytokine-induced signaling responses in cells. DNA Cell Biol. 16: 691-701.
2. Eberhardt, C., et al. 1997. Human lysophosphatidic acid acyltransferase. cDNA cloning, expression, and localization to chromosome 9q34.3. J. Biol. Chem. 272: 20299-20305.
3. Bursten, S.L. 1998. Interaction of lipopolysaccharide with a mammalian lyso-phosphatidate acyltransferase (LPAAT) transfected into *E. coli,* and effect of lisofylline on LPAAT transfected into mammalian cells. Prog. Clin. Biol. Res. 397: 345-356.
4. Aguado, B., et al. 1998. Characterization of a human lysophosphatidic acid acyltransferase that is encoded by a gene located in the class III region of the human major histocompatibility complex. J. Biol. Chem. 273: 4096-4105.
5. Eberhardt, C., et al. 1999. cDNA cloning, expression and chromosomal localization of two human lysophosphatidic acid acyltransferases. Adv. Exp. Med. Biol. 469: 351-356.
6. Yamashita, A., et al. 2001. ATP-independent fatty acyl-coenzyme A synthesis from phospholipid: coenzyme A-dependent transacylation activity toward lysophosphatidic acid catalyzed by acyl-coenzyme A:lysophosphatidic acid acyltransferase. J. Biol. Chem. 276: 26745-26752.

CHROMOSOMAL LOCATION

Genetic locus: AGPAT9 (human) mapping to 4q21.23.

PRODUCT

LPAAT-θ 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 LPAAT-θ shRNA Plasmid (h): sc-62565-SH and LPAAT-θ shRNA (h) Lentiviral Particles: sc-62565-V as alternate gene silencing products.

For independent verification of LPAAT-θ (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-62565A, sc-62565B and sc-62565C.

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

LPAAT-θ siRNA (h) is recommended for the inhibition of LPAAT-θ 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

LPAAT-θ (G-3): sc-514164 is recommended as a control antibody for monitoring of LPAAT-θ 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 LPAAT-θ gene expression knockdown using RT-PCR Primer: LPAAT-θ (h)-PR: sc-62565-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.