SANTA CRUZ BIOTECHNOLOGY, INC.

Ceramide Kinase siRNA (h): sc-72868

BACKGROUND

Ceramide Kinase, also known as CERK, LK4 (lipid kinase 4) or acylsphingosine kinase, is a 537 amino acid peripheral membrane protein that localizes to both the membrane and the cytoplasm and contains one DAGHc domain. Highly expressed in brain, liver, kidney, heart and skeletal muscle, with lower expression in spleen, lung, thymus and small intestine, Ceramide Kinase uses calcium and magnesium as cofactors to catalyze the ATP-dependent conversion of Ceramide to Ceramide 1-phosphate (C1P), a sphingolipid metabolite. Ceramide Kinase functions at an optimal pH of 6-7.5 and, via its catalytic activity, plays an important role in a variety of cellular processes, including apoptosis, phagocytosis and cellular proliferation.

REFERENCES

1. Sugiura, M., et al. 2002. Ceramide Kinase, a novel lipid kinase. Molecular cloning and functional characterization. J. Biol. Chem. 277: 23294-23300.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 610307. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Hinkovska-Galcheva, V., et al. 2005. Ceramide 1-phosphate, a mediator of phagocytosis. J. Biol. Chem. 280: 26612-26621.
4. Wijesinghe, D.S., et al. 2005. Substrate specificity of human Ceramide Kinase. J. Lipid Res. 46: 2706-2716.
5. Van Overloop, H., et al. 2006. Further characterization of mammalian Ceramide Kinase: substrate delivery and (stereo)specificity, tissue distribution, and subcellular localization studies. J. Lipid Res. 47: 268-283.
6. Mitra, P., et al. 2007. Ceramide Kinase regulates growth and survival of A549 human lung adenocarcinoma cells. FEBS Lett. 581: 735-740.
7. Date, T., et al. 2007. Ceramide Kinase expression is altered during macrophage-like cell differentiation of the leukemia cell line HL-60. In Vitro Cell. Dev. Biol. Anim. 43: 321-323.

CHROMOSOMAL LOCATION

Genetic locus: CERK (human) mapping to 22q13.31.

PRODUCT

Ceramide Kinase 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 Ceramide Kinase shRNA Plasmid (h): sc-72868-SH and Ceramide Kinase shRNA (h) Lentiviral Particles: sc-72868-V as alternate gene silencing products.

For independent verification of Ceramide Kinase (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-72868A, sc-72868B and sc-72868C.

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

Ceramide Kinase siRNA (h) is recommended for the inhibition of Ceramide Kinase 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

Ceramide Kinase (G-3): sc-376730 is recommended as a control antibody for monitoring of Ceramide Kinase 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 Ceramide Kinase gene expression knockdown using RT-PCR Primer: Ceramide Kinase (h)-PR: sc-72868-PR (20 µl, 590 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.