SANTA CRUZ BIOTECHNOLOGY, INC.

APHC siRNA (h): sc-96956

BACKGROUND

APHC (alkaline phytoceramidase), also known as ACER3 (alkaline ceramidase 3), PHCA (phytoceramidase, alkaline) or alkaline dihydroceramidase SB89, is a 267 amino acid multi-pass membrane protein that belongs to the alkaline ceramidase family and exists as 2 alternatively spliced isoforms. Encoded by a gene that maps to human chromosome 11q13.5, APHC is ubiquitously expressed, with highest expression in placenta, and localizes to endoplasmic reticulum and Golgi apparatus membranes. Activated by Ca^{2+} and inhibited by Zn^{2+}, APHC is conserved in chimpanzee, canine, bovine, mouse, rat, chicken, zebrafish, *Saccharomyces cerevisiae, Kluyveromyces lactis, Magnaporthe grisea, Neurospora crassa, Arabidopsis thaliana* and rice. APHC hydrolyzes phytoceramide into phytosphingosine and free fatty acid, but does not exhibit reverse activity. APHC also participates in hydrolase activity, acting on carbon-nitrogen bonds, but not peptide bonds, in linear amides.

REFERENCES

1. Kiepiela, P., et al. 2000. Genomic mutations in the katG, inhA and aphC genes are useful for the prediction of isoniazid resistance in *Mycobacterium tuberculosis* isolates from Kwazulu Natal, South Africa. Tuber. Lung Dis. 80: 47-56.
2. Mao, C., et al. 2001. Cloning and characterization of a novel human alkaline ceramidase. A mammalian enzyme that hydrolyzes phytoceramide. J. Biol. Chem. 276: 26577-26588.
3. Okamoto, S., et al. 2004. A phytochrome-like protein AphC triggers the cAMP signaling induced by far-red light in the cyanobacterium *Anabaena sp.* strain PCC7120. Photochem. Photobiol. 80: 429-433.
4. Mao, C. and Obeid, L.M. 2008. Ceramidases: regulators of cellular responses mediated by ceramide, sphingosine, and sphingosine-1-phosphate. Biochim. Biophys. Acta 1781: 424-434.
5. Yang, Q., et al. 2010. Role of *Drosophila* alkaline ceramidase (Dacer) in *Drosophila* development and longevity. Cell. Mol. Life Sci. 67: 1477-1490.
6. Xu, R., et al. 2010. Role of alkaline ceramidases in the generation of sphingosine and its phosphate in erythrocytes. FASEB J. 24: 2507-2515.

CHROMOSOMAL LOCATION

Genetic locus: ACER3 (human) mapping to 11q13.5.

PRODUCT

APHC siRNA (h) is a pool of 2 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 APHC shRNA Plasmid (h): sc-96956-SH and APHC shRNA (h) Lentiviral Particles: sc-96956-V as alternate gene silencing products.

For independent verification of APHC (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-96956A and sc-96956B.

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

APHC siRNA (h) is recommended for the inhibition of APHC 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor APHC gene expression knockdown using RT-PCR Primer: APHC (h)-PR: sc-96956-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.