SANTA CRUZ BIOTECHNOLOGY, INC.

ASAHL siRNA (h): sc-88929

BACKGROUND

ASAHL (N-acylsphingosine amidohydrolase (acid ceramidase)-like), also known as PLT or NAAA (N-acylethanolamine-hydrolyzing acid amidase), is a member of the choloylglycine hydrolase family and is widely expressed with predominant levels found in kidney and liver. ASAHL is structurally and functionally similar to Acid Ceramidase but exhibits low ceramide-hydrolyzing activity. Localizing to lysosomes, ASAHL functions in the hydrolyzation of bioactive N-acylethanolamines (NAEs) to ethanolamine and free fatty acids. Unlike FAAH (another NAE-hydrolyzing enzyme), ASAHL operates at an optimal pH of 4.5-5 and, once cleaved to its active form, exhibits a preference for N-palmitoylethanolamine and anandamide (N-arachidonoylethanolamine). ASAHL contains four glycosylation sites that are essential for stabilization of the enzyme and its activity is activated by dithiothreitol (DTT) and Triton X-100.

REFERENCES

1. Hong, S.B., et al. 1999. Molecular cloning and characterization of a human cDNA and gene encoding a novel Acid Ceramidase-like protein. Genomics 62: 232-241.
2. Puffenbarger, R.A. 2005. Molecular biology of the enzymes that degrade endocannabinoids. Curr. Drug Targets CNS Neurol. Disord. 4: 625-631.
3. Sun, Y.X., et al. 2005. Involvement of N-acylethanolamine-hydrolyzing acid amidase in the degradation of anandamide and other N-acylethanolamines in macrophages. Biochim. Biophys. Acta 1736: 211-220.
4. Tsuboi, K., et al. 2005. Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to Acid Ceramidase. J. Biol. Chem. 280: 11082-11092.
5. Astarita, G., et al. 2006. Pharmacological characterization of hydrolysis-resistant analogs of oleoylethanolamide with potent anorexiant properties. J. Pharmacol. Exp. Ther. 318: 563-570.
6. Tsuboi, K., et al. 2007. The N-acylethanolamine-hydrolyzing acid amidase (NAAA). Chem. Biodivers. 4: 1914-1925.

CHROMOSOMAL LOCATION

Genetic locus: NAAA (human) mapping to 4q21.1.

PRODUCT

ASAHL 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 ASAHL shRNA Plasmid (h): sc-88929-SH and ASAHL shRNA (h) Lentiviral Particles: sc-88929-V as alternate gene silencing products.

For independent verification of ASAHL (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-88929A and sc-88929B.

RESEARCH USE

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

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

ASAHL siRNA (h) is recommended for the inhibition of ASAHL 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

ASAHL (A19): sc-100470 is recommended as a control antibody for monitoring of ASAHL 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 ASAHL gene expression knockdown using RT-PCR Primer: ASAHL (h)-PR: sc-88929-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.