

SAHH siRNA (h): sc-62972

BACKGROUND

SAHH (S-adenosylhomocysteine hydrolase), also known as AHCY, is a 432 amino acid cytoplasmic protein that localizes to the melanosome, a melanin-containing organelle. An essential component of amino acid biosynthesis, SAHH catalyzes the reversible hydrolysis of S-adenosylhomocysteine (SAH) to produce adenosine and L-homocysteine. Through its catalytic activity, SAHH regulates the intracellular levels of SAH and may play a key role in controlling methyltransferase reactions. SAHH exists as a homotetramer that can bind one NAD per subunit and is involved in the activated methyl cycle (a reaction in which the methyl group of methionine is activated by the formation of S-adenosylmethionine). Defects in the gene encoding SAHH result in elevated levels of methionine which cause hypermethioninemia, a disease characterized by sluggishness, muscle weakness and liver problems.

REFERENCES

1. Elrod, P., et al. 2002. Contributions of active site residues to the partial and overall catalytic activities of human S-adenosylhomocysteine hydrolase. *Biochemistry* 41: 8134-8142.
2. Yang, X., et al. 2003. Catalytic strategy of S-adenosylhomocysteine hydrolase: transition-state stabilization and the avoidance of abortive reactions. *Biochemistry* 42: 1900-1909.
3. Kloor, D. and Osswald, H. 2004. S-adenosylhomocysteine hydrolase as a target for intracellular adenosine action. *Trends Pharmacol. Sci.* 25: 294-297.
4. Shu, S., et al. 2006. S-adenosylhomocysteine hydrolase is localized at the front of chemotaxing cells, suggesting a role for transmethylation during migration. *Proc. Natl. Acad. Sci. USA* 103: 19788-19793.
5. Hermes, M., et al. 2007. Role of S-adenosylhomocysteine hydrolase in adenosine-induced apoptosis in HepG2 cells. *Exp. Cell Res.* 313: 264-283.

CHROMOSOMAL LOCATION

Genetic locus: AHCY (human) mapping to 20q11.22.

PRODUCT

SAHH 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SAHH shRNA Plasmid (h): sc-62972-SH and SAHH shRNA (h) Lentiviral Particles: sc-62972-V as alternate gene silencing products.

For independent verification of SAHH (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-62972A, sc-62972B and sc-62972C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

SAHH siRNA (h) is recommended for the inhibition of SAHH 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 μ M in 66 μ l. 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

SAHH (A-11): sc-271389 is recommended as a control antibody for monitoring of SAHH 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 SAHH gene expression knockdown using RT-PCR Primer: SAHH (h)-PR: sc-62972-PR (20 μ l). 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.