SANTA CRUZ BIOTECHNOLOGY, INC.

SIAH-1/2 siRNA (h): sc-44102

BACKGROUND

SIAH, the human homolog of the *Drosophila* seven in absentia (sina) gene, is a tumor suppressor protein that is expressed in intestinal epithelium and activated during apoptosis. Human SIAH proteins are produced as two distinct gene products, SIAH-1, and the slightly larger protein SIAH-2, which share a highly conserved C-terminal sequence and differ in their N-terminal regions. SIAH-1 contains an N-terminal RING-finger domain, which is required for proteolysis, and a cystein-rich C-terminal domain, which regulates oligomerization and SIAH binding to target proteins. As a tumor suppressor, SIAH-1 binds DCC (deleted in colorectal cancer) and regulates DCC degradation via the ubiquitin-proteasome pathway. SIAH-1 also binds a Bcl-2 related protein, Bag-1, thereby inhibiting cell growth. The majority of SIAH-1 is localized to the nucleus, however a small percentage is detected in the cytoplasm. This nuclear localization suggests that SIAH proteins may interact with other nuclear matrix proteins and DNA.

REFERENCES

1. Nemani, M., et al. 1996. Activation of the human homologue of the *Drosophila* sina gene in apoptosis and tumor suppression. Proc. Natl. Acad. Sci. USA 93: 9039-9042.
2. Hu, G., et al. 1997. Mammalian homologs of seven in absentia regulate DCC via the ubiquitin-proteasome pathway. Genes Dev. 11: 2701-2714.
3. Hu, G., et al. 1997. Characterization of human homologs fo the *Drosophila* seven in absentia (sina) gene. Genomics 46: 103-111.
4. Matsuzawas, S., et al. 1998. P53-inducible human homologue of *Drosophila* seven in absentia (Siah) inhibits cell growth: suppression by BAG-1. EMBO J. 17: 2736-2747.
5. Hu, G., et al. 1999. Siah-1 N-terminal RING domain is required for proteolysis function, and C-terminal sequence regulate oligomerization and binding to target proteins. Mol. Cell. Biol. 19: 724-732.
6. Roperch, J., et al. 1999. SIAH-1 promotes apoptosis and tumor suppression through a network involving the regulation of protein folding, unfolding, and trafficking: identification of common effectors with p53 and $p21^{Waf1}$. Proc. Natl. Acad. Sci. USA 96: 8070-8073.

CHROMOSOMAL LOCATION

Genetic locus: SIAH1 (human) mapping to 16q12.1, SIAH2 (human) mapping to 3q25.1.

PRODUCT

SIAH-1/2 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 SIAH-1/2 shRNA Plasmid (h): sc-44102-SH and SIAH-1/2 shRNA (h) Lentiviral Particles: sc-44102-V as alternate gene silencing products.

For independent verification of SIAH-1/2 (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-44102A, sc-44102B and sc-44102C.

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

SIAH-1/2 siRNA (h) is recommended for the inhibition of SIAH-1/2 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

SIAH-1/2 (8G7H12): sc-81785 is recommended as a control antibody for monitoring of SIAH-1/2 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SIAH-1/2 gene expression knockdown using RT-PCR Primer: SIAH-1/2 (h)-PR: sc-44102-PR (20 µl, 584 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Krämer, O.H., et al. 2008. Mechanism for ubiquitylation of the leukemia fusion proteins AML1-ETO and PML-RARα. FASEB J. 22: 1369-1379.
2. Buchwald, M., et al. 2012. SIAH ubiquitin ligases target the nonreceptor tyrosine kinase ACK1 for ubiquitinylation and proteasomal degradation. Oncogene 32: 4913-4920.

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.