SANTA CRUZ BIOTECHNOLOGY, INC.

Shh siRNA (h): sc-29477

BACKGROUND

The *Drosophila* segment polarity gene hedgehog (hh) encodes a precursor protein which undergoes autocleavage to generate amino and carboxy terminal peptides. Both proteins are secreted and appear to function in embryonic and imaginal disc patterning. Several vertebrate homologs of *Drosophila* hh have been identified. These include Sonic hedgehog (Shh) (alternatively designated Vhh-1), Desert hedgehog (Dhh) and Indian hedgehog (Ihh). Each contain amino terminal signal peptides and apparently function as secreted proteins involved in the mediation of various cell-cell interactions. Shh resembles *Drosophila* hh in that it is processed to generate an amino terminal secreted peptide that is retained at or near the cell surface and a carboxy terminal glycosylated more diffusible peptide.

REFERENCES

1. Echelard, Y., et al. 1993. Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell 75: 1417-1430.
2. Li, W., et al. 1995. Function of protein kinase A in hedgehog signal transduction and *Drosophila* imaginal disc development. Cell 80: 553-562.
3. Johnson, R.L., et al. 1995. The long and short of hedgehog signaling. Cell 81: 313-316.

CHROMOSOMAL LOCATION

Genetic locus: SHH (human) mapping to 7q36.3.

PRODUCT

Shh 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 Shh shRNA Plasmid (h): sc-29477-SH and Shh shRNA (h) Lentiviral Particles: sc-29477-V as alternate gene silencing products.

For independent verification of Shh (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-29477A, sc-29477B and sc-29477C.

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

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

Shh (E-1): sc-365112 is recommended as a control antibody for monitoring of Shh 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 Shh gene expression knockdown using RT-PCR Primer: Shh (h)-PR: sc-29477-PR (20 µl, 560 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Chang, S.C., et al. 2011. Two distinct sites in Sonic hedgehog combine for heparan sulfate interactions and cell signaling functions. J. Biol. Chem. 286: 44391-44402.
2. Jiang, K., et al. 2017. Fms related tyrosine kinase 1 (Flt1) functions as an oncogene and regulates glioblastoma cell metastasis by regulating Sonic hedgehog signaling. Am. J. Cancer Res. 7: 1164-1176.
3. Qu, W., et al. 2018. Activation of Sonic hedgehog signaling is associated with human osteosarcoma cells radioresistance characterized by increased proliferation, migration, and invasion. Med. Sci. Monit. 24: 3764-3771.
4. Wei, M., et al. 2019. Oroxylin A increases the sensitivity of temozolomide on glioma cells by hypoxia-inducible factor 1α/hedgehog pathway under hypoxia. J. Cell. Physiol. 234: 17392-17404.
5. Bissey, P.A., et al. 2020. Blocking SHH/patched interaction triggers tumor growth inhibition through patched-induced apoptosis. Cancer Res. 80: 1970-1980.

RESEARCH USE

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