

HHIPL1 siRNA (h): sc-92168

BACKGROUND

Hedgehog (Hh) signaling proteins are critical for growth and tissue patterning during development. Patched (Ptc), a putative 12 transmembrane receptor, binds to Sonic hedgehog and is suspected to be a negative regulator of Hh signaling. A family member of patched, designated patched 2, has been found to be co-expressed with Sonic hedgehog. Smoothened (Smo), a seven transmembrane receptor, is complexed with patched in many tissues and is believed to be an essential component in the Hh signaling pathway. Hhip (hedgehog-interacting protein) is able to bind to and may be a transcriptional target of all Hh proteins. Binding of Hhip to Hh proteins attenuates Hedgehog signaling. HHIPL1 (hedgehog-interacting protein-like protein 1), also known as HHIP2, is a 782 amino acid secreted protein that contains a HHIP domain and is ex-pressed in trabecular bone. There are two isoforms of HHIPL1 that are produced as a result of alternative splicing events.

REFERENCES

1. Katoh, Y. and Katoh, M. 2006. Comparative genomics on HHIP family orthologs. *Int. J. Mol. Med.* 17: 391-395.
2. Choi, S.S., et al. 2009. Hedgehog pathway activation and epithelial-to-mesenchymal transitions during myofibroblastic transformation of rat hepatic cells in culture and cirrhosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 297: G1093-G1106.
3. Eichenmüller, M., et al. 2009. Blocking the hedgehog pathway inhibits hepatoblastoma growth. *Hepatology* 49: 482-490.
4. Bosanac, I., et al. 2009. The structure of SHH in complex with HHIP reveals a recognition role for the Shh pseudo active site in signaling. *Nat. Struct. Mol. Biol.* 16: 691-697.
5. Bishop, B., et al. 2009. Structural insights into hedgehog ligand sequestration by the human hedgehog-interacting protein HHIP. *Nat. Struct. Mol. Biol.* 16: 698-703.
6. Beachy, P.A., et al. 2010. Interactions between Hedgehog proteins and their binding partners come into view. *Genes Dev.* 24: 2001-2012.
7. Shahi, M.H., et al. 2010. Human hedgehog interacting protein expression and promoter methylation in medulloblastoma cell lines and primary tumor samples. *J. Neurooncol.* 103: 287-296.

CHROMOSOMAL LOCATION

Genetic locus: HHIPL1 (human) mapping to 14q32.2.

PRODUCT

HHIPL1 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 HHIPL1 shRNA Plasmid (h): sc-92168-SH and HHIPL1 shRNA (h) Lentiviral Particles: sc-92168-V as alternate gene silencing products.

For independent verification of HHIPL1 (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-92168A, sc-92168B and sc-92168C.

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

HHIPL1 siRNA (h) is recommended for the inhibition of HHIPL1 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

HHIPL1 (H-11): sc-515618 is recommended as a control antibody for monitoring of HHIPL1 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 HHIPL1 gene expression knockdown using RT-PCR Primer: HHIPL1 (h)-PR: sc-92168-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.

PROTOCOLS

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