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Hhip siRNA (h): sc-43835

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. Smoothed (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.

REFERENCES

1. Stone, D.M., et al. 1996. The tumour-suppressor gene patched encodes a candidate receptor for Sonic hedgehog. *Nature* 384: 129-134.
2. Goodrich, L.V., et al. 1996. Conservation of the hedgehog/patched signaling pathway from flies to mice: induction of a mouse patched gene by Hedgehog. *Genes Dev.* 10: 301-312.
3. Marigo, V., et al. 1996. Regulation of patched by Sonic hedgehog in the developing neural tube. *Proc. Natl. Acad. Sci. USA* 93: 9346-9351.
4. Weed, M., et al. 1997. The role of Sonic hedgehog in vertebrate development. *Matrix Biol.* 16: 53-58.
5. Motoyama, J., et al. 1998. Ptch2, a second mouse patched gene is coexpressed with Sonic hedgehog. *Nat. Genet.* 18: 104-106.
6. Chuang, P.T., et al. 1999. Vertebrate Hedgehog signalling modulated by induction of a hedgehog-binding protein. *Nature* 397: 617-621.

CHROMOSOMAL LOCATION

Genetic locus: HHIP (human) mapping to 4q31.21.

PRODUCT

Hhip 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 Hhip shRNA Plasmid (h): sc-43835-SH and Hhip shRNA (h) Lentiviral Particles: sc-43835-V as alternate gene silencing products.

For independent verification of Hhip (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-43835A, sc-43835B and sc-43835C.

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

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

Hhip (5D11): sc-293265 is recommended as a control antibody for monitoring of Hhip 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 Hhip gene expression knockdown using RT-PCR Primer: Hhip (h)-PR: sc-43835-PR (20 μ l, 599 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ 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.