

# patched siRNA (h): sc-36192

## BACKGROUND

Overexpression of either Wnt-1 or the GLI proteins have been shown to result in cancer. These proteins exist in a signal cascade downstream of the mammalian homologs of the *Drosophila* hedgehog (hh) and patched (ptc) proteins. The hedgehog protein mediates embryonic and imaginal disc patterning, and patched expression is precisely regulated during embryonic development. Hedgehog enhances the expression of the WNT family of proteins through a signaling cascade involving the GLI transcription factors, while patched functions as a repressor opposing hedgehog's effects. Mutations in the ptc gene, which result in unregulated hedgehog signaling, correlates with the most common type of cancer, basal cell carcinoma, which affects 750,000 individuals annually in the United States. An additional patched family member, patched 2, has been found to be coexpressed with Sonic hedgehog.

## REFERENCES

1. Nusslein-Volhard, C., et al. 1980. Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287: 795-801.
2. Kinzler, K.W., et al. 1987. Identification of an amplified, highly expressed gene in a human glioma. *Science* 236: 70-73.
3. Parkin, N.T., et al. 1993. Activity of Wnt-1 as a transmembrane protein. *Genes Dev.* 7: 2181-2193.
4. Marti, E., et al. 1995. Requirement of 19K form of sonic hedgehog for induction of distinct ventral cell types in CNS explants. *Nature* 375: 322-325.

## CHROMOSOMAL LOCATION

Genetic locus: PTCH1 (human) mapping to 9q22.32.

## PRODUCT

patched 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see patched shRNA Plasmid (h): sc-36192-SH and patched shRNA (h) Lentiviral Particles: sc-36192-V as alternate gene silencing products.

For independent verification of patched (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-36192A, sc-36192B and sc-36192C.

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

## APPLICATIONS

patched siRNA (h) is recommended for the inhibition of patched 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  $\mu$ M in 66  $\mu$ 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

patched (A-2): sc-518044 is recommended as a control antibody for monitoring of patched 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor patched gene expression knockdown using RT-PCR Primer: patched (h)-PR: sc-36192-PR (20  $\mu$ l, 596 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Agouni, A., et al. 2007. Sonic hedgehog carried by microparticles corrects endothelial injury through nitric oxide release. *FASEB J.* 21: 2735-2741.
2. Soleti, R., et al. 2009. Microparticles harboring Sonic Hedgehog promote angiogenesis through the upregulation of adhesion proteins and proangiogenic factors. *Carcinogenesis* 30: 580-588.
3. 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.