SignalSilence® Sharpin siRNA I

#12875 Store at -20°C

✔ 10 µM in 300 µl
(3 nmol)

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For Research Use Only. Not For Use In Diagnostic Procedures.

Entrez-Gene ID #81858
Swiss-Prot Acc. #Q9HOF6

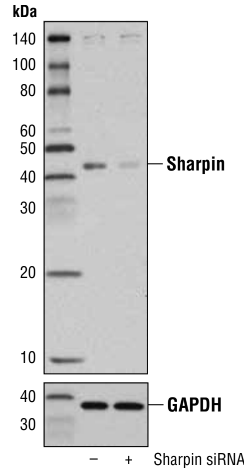
Species Cross-Reactivity: H

Description: SignalSilence® Sharpin siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit sharpin expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

Background: SHank-Associated RH domain-interacting ProteIN (*Sharpin*), also known as *SIPL1*, is a highly conserved gene among many mammalian species and is ubiquitously expressed in various types of cells and tissues. Sharpin harbors multiple functional motifs including an amino terminal coiled-coil (CC) domain, which has been shown to mediate the interaction between sharpin and the scaffold protein shank (1). The other two domains, ubiquitin-like domain (UBL) and NPL4 zinc finger domain (NZF), facilitate ubiquitin-mediated protein recognition and degradation (2). Recent studies have shown that both UBL and NZF domains are essential for sharpin to exert its function in part through ubiquitin-mediated mechanisms (3-5). Although sharpin was initially identified as a scaffold protein within the postsynaptic density of neurons (1), recent studies have identified sharpin as a novel modulator of immune and inflammatory diseases. An emerging mechanistic model suggests that sharpin functions as an important adaptor component of the linear ubiquitin chain assembly complex (LUBAC) that modulates activation of the canonical NF-κB signaling pathway (3,4,6,7), thereby regulating cell survival and apoptosis, cytokine production, and development of lymphoid tissues. Indeed, mice with spontaneous mutations in the *sharpin* gene develop chronic proliferative dermatitis that is characterized by eosinophilic inflammation of the skin and dysregulated development of lymphoid tissues (8).

Directions for Use: CST recommends transfection with 100 nM SignalSilence® Sharpin siRNA I 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.

Each vial contains the equivalent of 100 transfections, which corresponds to a final siRNA concentration of 100 nM per transfection in a 24-well plate with a total volume of 300 µl per well.



Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® Sharpin siRNA I (+), using Sharpin (D4P5B) Rabbit mAb #12541 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The Sharpin (D4P5B) Rabbit mAb confirms silencing of sharpin expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

Quality Control: Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.

Storage: Sharpin siRNA I is supplied in RNAse-free water. *Aliquot and store at -20ºC.*

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Lim, S. et al. (2001) *Mol Cell Neurosci* 17, 385-97.
(2) Grabbe, C. and Dikic, I. (2009) *Chem Rev* 109, 1481-94.
(3) Ikeda, F. et al. (2011) *Nature* 471, 637-41.
(4) Tokunaga, F. et al. (2011) *Nature* 471, 633-6.
(5) Iwai, K. (2011) *Cell Cycle* 10, 3095-104.
(6) Gerlach, B. et al. (2011) *Nature* 471, 591-6.
(7) Tokunaga, F. et al. (2009) *Nat Cell Biol* 11, 123-32.
(8) Seymour, R.E. et al. (2007) *Genes Immun* 8, 416-21.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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