SignalSilence® B-Raf siRNA I

#8935 Store at -20°C

☑ 10 µM in 300 µl (3 nmol)

Cell Signaling TECHNOLOGY®

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

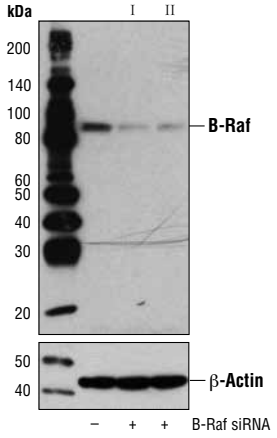
Species Cross-Reactivity: H, (Mk)

Description: SignalSilence® B-Raf siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit B-Raf 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: A-Raf, B-Raf, and c-Raf (Raf-1) are the main effectors recruited by GTP-bound Ras to activate the MEK-MAP kinase pathway (1). Activation of c-Raf is the best understood and involves phosphorylation at multiple activating sites including Ser338, Tyr341, Thr491, Ser494, Ser497, and Ser499 (2). p21-activated protein kinase (PAK) has been shown to phosphorylate c-Raf at Ser338 and the Src family phosphorylates Tyr341 to induce c-Raf activity (3,4). Ser338 of c-Raf corresponds to similar sites in A-Raf (Ser299) and B-Raf (Ser445), although this site is constitutively phosphorylated in B-Raf (5). Inhibitory 14-3-3 binding sites on c-Raf (Ser259 and Ser621) can be phosphorylated by Akt and AMPK, respectively (6,7). While A-Raf, B-Raf, and c-Raf are similar in sequence and function, differential regulation has been observed (8). Of particular interest, B-Raf contains three consensus Akt phosphorylation sites (Ser364, Ser428, and Thr439) and lacks a site equivalent to Tyr341 of c-Raf (8,9). Research studies have shown that the B-Raf mutation V600E results in elevated kinase activity and is commonly found in malignant melanoma (10). Six residues of c-Raf (Ser29, Ser43, Ser289, Ser296, Ser301, and Ser642) become hyperphosphorylated in a manner consistent with c-Raf inactivation. The hyperphosphorylation of these six sites is dependent on downstream MEK signaling and renders c-Raf unresponsive to subsequent activation events (11).

Specificity/Sensitivity: B-Raf siRNA I inhibits human and monkey B-Raf expression.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® B-Raf 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 HT-29 cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® B-Raf siRNA I (+), or SignalSilence® B-Raf siRNA II #9439 (+), using B-Raf (55C6) Rabbit mAb #9433 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The B-Raf (55C6) Rabbit mAb confirms silencing of B-Raf expression, while the β-Actin (D6A8) 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.

Entrez-Gene ID #673
Swiss-Prot Acc. #P15056

Storage: B-Raf siRNA I is supplied in RNAse-free water. *Aliquot and store at -20°C.*

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

Background References:

(1) Avruch, J. et al. (1994) *Trends Biochem. Sci.* 19, 279-283.
(2) Chong, H. et al. (2001) *EMBO J.* 20, 3716-3727.
(3) King, A.J. et al. (1998) *Nature* 396, 180-183.
(4) Fabian, J.R. et al. (1993) *Mol. Cell Biol.* 13, 7170-7179.
(5) Mason, C.S. et al. (1999) *EMBO J.* 18, 2137-2148.
(6) Zimmermann, S. and Moelling, K. (1999) *Science* 286, 1741-1744.
(7) Sprenkle, A.B. et al. (1997) *FEBS Lett.* 403, 254-258.
(8) Marais, R. et al. (1997) *J. Biol. Chem.* 272, 4378-4383.
(9) Guan, K.L. et al. (2000) *J. Biol. Chem.* 275, 27354-27359.
(10) Davies, H. et al. (2002) *Nature* 417, 949-954.
(11) Dougherty, M.K. et al. (2005) *Mol. Cell* 17, 215-224.

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.