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SignalSilence® Bub1b siRNA I



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

Species Cross-Reactivity: H, (Mk)

Description: SignalSilence® Bub1b siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit Bub1b 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.

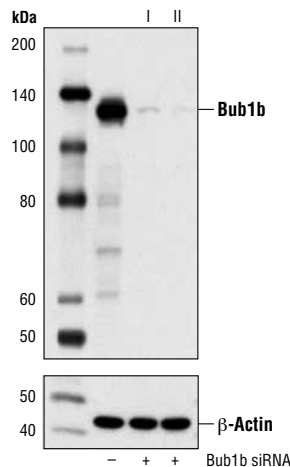
Background: The Mitotic Checkpoint Complex (MCC), which contains Bub1, Bub1b, Bub3, Mad2, and Cdc20, controls chromosome segregation and monitors kinetochore-microtubule interactions (1). During mitosis, the MCC complex inhibits the ubiquitin ligase activity of the Anaphase Promoting Complex/Cyclosome (APC/C), thereby preventing cells with unaligned chromosomes from prematurely entering anaphase (2). Research studies have shown that Bub1b and Bub1 kinases are mutated in several types of human malignancies including hematopoietic, colorectal, lung, and breast cancers (3). Biallelic mutations in Bub1b have been found in mosaic variegated aneuploidy syndrome and premature chromatid separation syndrome (4). Bub1b mouse germline knockouts are embryonic lethal with heterozygous animals displaying genetic instability, early aging phenotypes, and increased cancer susceptibility (5). Bub3 binds both Bub1 and Bub1b, facilitating their recruitment to kinetochores (6), and is required for functional microtubule-kinetochore interactions (7).

Specificity/Sensitivity: SignalSilence® Bub1b siRNA I inhibits human and monkey Bub1b expression.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® Bub1b 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.

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.



Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® Bub1b siRNA I (+), or SignalSilence® Bub1b siRNA II #12150 (+), using Bub1b (D32E8) Rabbit mAb #5421 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The Bub1b (D32E8) Rabbit mAb confirms silencing of Bub1b expression, while the β-Actin (D6A8) Rabbit mAb is used as a loading control.

Entrez-Gene ID #701
Swiss-Prot Acc. #060566

Storage: Bub1b 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) Fukagawa, T. (2008) *Front. Biosci.* 13, 2705–2713.
- (2) Chen, R.H. (2007) *J. Biomed. Sci.* 14, 475–479.
- (3) Dai, W. et al. (2004) *Cancer Res.* 64, 440–445.
- (4) Kops, G.J. et al. (2005) *Nat. Rev. Cancer* 5, 773–785.
- (5) Baker, D.J. et al. (2004) *Nat. Genet.* 36, 744–749.
- (6) Taylor, S.S. et al. (1998) *J. Cell. Biol.* 142, 1–11.
- (7) Logarinho, E. et al. (2008) *Mol. Biol. Cell* 19, 1798–1813.

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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.