SignalSilence® BRCA1 siRNA I

10 μM in 300 μl
(3 nmol)

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

Species Cross-Reactivity: H

Description: SignalSilence[®] BRCA1 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit BRCA1 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 breast cancer susceptibility proteins BRCA1 and BRCA2 are frequently mutated in cases of hereditary breast and ovarian cancers and have roles in multiple processes related to DNA damage, repair, cell cycle progression, transcription, ubiquitination, and apoptosis (1-4). BRCA2 has been shown to be required for localization of Rad51 to sites of double stranded breaks (DSBs) in DNA, and cells lacking BRCA1 and BRCA2 cannot repair DSBs through the Rad51-dependent process of homologous recombination (HR) (5). Numerous DNA damage-induced phosphorylation sites on BRCA1 have been identified, including Ser988, 1189, 1387, 1423, 1457, 1524 and 1542, and kinases activated in a cell cycle-dependent manner, including Aurora A and CDK2, can also phosphorylate BRCA1 at Ser308 and Ser1497, respectively (6-10). Cell cycle-dependent phosphorylation of BRCA2 at Ser3291 by CDKs has been proposed as a mechanism to switch off HR as cells progress beyond S-phase by blocking the carboxy terminal Rad51 binding site (11).

Directions for Use: CST recommends transfection with 100 nM SignalSilence® BRCA1 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.



rev. 05/19/16

Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® BRCA1 siRNA I (+), or SignalSilence® BRCA1 siRNA II #12642 (+), using BRCA1 Antibody #9010 (upper) or β -Actin (D6A8) Rabbit mAb #8457 (lower). The BRCA1 Antibody confirms silencing of BRCA1 expression, while the β -Actin (D6A8) Rabbit mAb is used as a loading control.



OrdersImage: 877-616-CELL (2355)
orders@cellsignal.comSupportImage: 877-678-TECH (8324)
info@cellsignal.comWebImage: 800 model

Entrez-Gene ID #672 Swiss-Prot Acc. #P38398

Storage: SignalSilence[®] siRNA is supplied in RNAse-free water. Aliquot and store at -20 $^{\circ}$ C.

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

Background References:

- (1) Rahman, N. and Stratton, M.R. (1998) *Annu. Rev. Genet.* 32, 95-121.
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- (4) Scully, R. and Livingston, D.M. (2000) Nature 408, 429-432.
- (5) Tutt, A. and Ashworth, A. (2002) *Trends Mol. Med.* 8, 571-576.
- (6) Okada, S. and Ouchi, T. (2003) J. Biol. Chem. 278, 2015-2020.

(7) Cortez, D. et al. (1999) Science 286, 1162-1166.

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(9) Ouchi, M. et al. (2004) J. Biol. Chem. 279, 19643-19648.

(10) Ruffner, H. et al. (1999) *Mol. Cell. Biol.* 19, 4843-4854.

(11) Esashi, F. et al. (2005) *Nature* 434, 598-604.

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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 Dp—dop Pp—nin Sp—S cerevisiae Ce—C, elenans Hr—Horse AII—all species exocuted Species enclosed in parentheses are predicted to react based on 100% homology.