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SignalSilence® RAP80 siRNA I

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Entrez-Gene ID #51720 UniProt ID #Q96RL1

New 10/14

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

Species Cross-Reactivity: H

Description: SignalSilence® RAP80 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit RAP80 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.

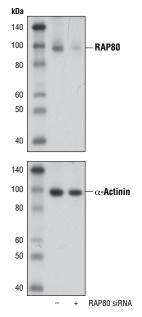
Background: The breast cancer type 1 susceptibility protein (BRCA1) is an E3 ubiquitin ligase that functions in the maintenance of genome stability through regulation of the DNA damage response and DNA repair. BRCA1 protein forms at least three distinct complexes (BRCA1 A, B, and C) with other DNA repair proteins, and these interactions are vital for regulation of BRCA1 protein function. The BRCA1-RAP80 complex (BRCA1 A complex) includes RAP80, BRCC36, BRE, Abraxas, and NBA1 and functions in G2/M phase checkpoint control (reviewed in 1,2).

The ubiquitously expressed receptor-associated protein 80 (RAP80, UIMC1) is required for recruitment and stability of the BRCA1 A complex at sites of DNA damage (3). Research studies indicate that the absence of RAP80 in cells results in increased sensitivity to the topoisomerase II inhibitor etoposide (4). In the absence of functional RAP80, BRCA1 A complex function is suppressed and cells become more sensitive to DNA damage-induced genome instability (5,6). Phosphorylation of RAP80 by CDK1/Cyclin B at Ser177 regulates RAP80 function at the mitotic checkpoint (7). A naturally occurring in-frame deletion mutant within RAP80 likely alters RAP80 protein-protein interactions and is associated with an increase in chromosomal abnormalities (8,9).

Directions for Use: CST recommends transfection with 100 nM RAP80 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 μ l 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 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® RAP80 siRNA I (+), using RAP80 (D1T6Q) Rabbit mAb #14466 (upper) and α -Actinin (D6F6) XP® Rabbit mAb #6487 (lower). The RAP80 (D1T6Q) Rabbit mAb confirms silencing of RAP80 expression, while the α -Actinin (D6F6) XP® Rabbit mAb is used as a loading control.

Storage: RAP80 siRNA I is supplied in RNAse-free water. *Aliquot and store at -20°C*.

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background References:

- (1) Ohta, T. et al. (2011) FEBS Lett 585, 2836-44.
- (2) Huen, M.S. et al. (2010) Nat Rev Mol Cell Biol 11, 138-48.
- (3) Wu, J. et al. (2012) J Biol Chem 287, 22919-26.
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- (5) Bian, C. et al. (2012) PLoS One 7, e40406.
- (6) Yin, Z. et al. (2012) Cancer Res 72, 5080-90.
- (7) Cho, H.J. et al. (2013) J Biol Chem 288, 3768-76.
- (8) Nikkilä, J. et al. (2009) Oncogene 28, 1843-52.
- (9) Anamika et al. (2014) J Biol Chem 289, 12852-62.

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