SignalSilence® OTULIN siRNA I

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

Species Cross-Reactivity: H

Description: SignalSilence® OTULIN siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit OTULIN 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: Protein ubiquitination and deubiquitination are reversible processes catalyzed by ubiquitinating enzymes (UBEs) and deubiquitinating enzymes (DUBs) (1,2). Five subfamilies of DUBs have been characterized to date, and include USP, UCH, OTU, MJD, and JAMM deubiquitinating enzymes (1,2). The ovarian tumor (OTU) DUB subfamily comprises a group of approximately 100 putative cysteine proteases that are homologous to the Drosophila ovarian tumor gene product (3). OTU domain-containing deubiquitinase with linear linkage specificity (OTULIN, FAM105B, Gumby) is an OTU subfamily deubiguitinating enzyme that antagonizes the E3 linear ubiguitin chain assembly complex (LUBAC) by promoting disassembly of Met1-linked (linear) ubiquitin chains (4.5), LUBAC and OTULIN regulate NOD2 signaling in an antagonistic manner by controlling the level of Met1-ubiquitinated RIPK2 kinase (6). Binding of the OTULIN PUB-interacting motif to the HOIP subunit of LUBAC is critical for OTULIN inhibition of NF-**kB** signaling; this OTULIN-HOIP interaction is negatively regulated by tyrosine phosphorylation of OTULIN (7,8). The ability of OTULIN to influence LUBAC function and the presence of linear ubiquitin chains may play an important role in regulating angiogenesis, craniofacial, and neural development (5).

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



kDa 200

140

New 07/14

Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® OTULIN siRNA I (+), using OTULIN Antibody #14127 (upper) and GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The OTULIN Antibody confirms silencing of OTULIN expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

www.cellsignal.com

Support: 877-678-TECH (8324) info@cellsianal.com

> Orders: 877-616-CELL (2355) orders@cellsignal.com

> Entrez-Gene ID #90268 UniProt ID #Q96BN8

Storage: OTULIN 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:

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Nalepa, G. et al. (2006) *Nat Rev Drug Discov* 5, 596-613.
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Keusekotten, K. et al. (2013) *Cell* 153, 1312-26.
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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: 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 AII—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.