**:6399** Store at -20°C

## SignalSilence® SQSTM1/p62 siRNA II

10 μM in 300 μl (100 Transfections)

rev. 02/10/16



## Species Cross-Reactivity: H, (M, R, Hm, Mk)

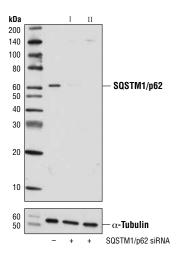
**Description:** SignalSilence<sup>®</sup> SQSTM1/p62 siRNA II from Cell Signaling Technology (CST) allows the researcher to specifically inhibit SQSTM1/p62 expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence<sup>®</sup> siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

Background: Sequestosome 1 (SQSTM1, p62) is a ubiquitin binding protein involved in cell signaling, oxidative stress, and autophagy (1-4). It was first identified as a protein that binds to the SH2 domain of p56Lck (5), and independently found to interact with PKC $\zeta$  (6,7). It was subsequently found to interact with ubiquitin, providing a scaffold for several signaling proteins and triggering degradation of proteins through the proteasome or lysosome (8). Interaction between SQSTM1 and TRAF6 leads to the K63-linked polyubiquitination of TRAF6 and subsequent activation of the NF-kB pathway (9). Protein aggregates formed by SQSTM1 can be degraded by the autophagosome (4,10,11). SQSTM1 binds autophagosomal membrane protein LC3/Atg8, bringing SQSTM1-containing protein aggregates to the autophagosome (12). Lysosomal degradation of autophagosomes leads to a decrease in SQSTM1 levels during autophagy; conversely, autophagy inhibitors stabilize SQSTM1 levels. Studies have demonstrated a link between SQSTM1 and oxidative stress. SQSTM1 interacts with KEAP1, which is a cytoplasmic inhibitor of NRF2, a key transcription factor involved in cellular responses to oxidative stress (3). Thus, accumulation of SQSTM1 can lead to an increase in NRF2 activity.

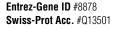
**Directions for Use:** CST recommends transfection with 100 nM SQSTM1/p62 siRNA II 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.

**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.

**Specificity/Sensitivity:** SQSTM1/p62 siRNA II inhibits human, mouse, rat, and monkey SQSTM1/p62 expression.



Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® SQSTM1/p62 siRNA I #6394 (+) or SignalSilence® SQSTM1/p62 siRNA II (+), using SQSTM1/p62 (D5E2) Rabbit mAb #8025 (upper) or  $\alpha$ -tubulin (11H10) Rabbit mAb #2125 (lower). The SQSTM1/p62 (D5E2) Rabbit mAb confirms silencing of SQSTM1/p62 expression, while the  $\alpha$ -Tubulin (11H10) Rabbit mAb is used as a loading control.



**Storage:** SQSTM1/p62 siRNA II 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) Kirkin, V. et al. (2009) Mol Cell 34, 259-69.

Cell Signaling

Orders 877-616-CELL (2355)

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(2) Seibenhener, M.L. et al. (2007) FEBS Lett 581, 175-9.

(3) Komatsu, M. et al. (2010) Nat Cell Biol 12, 213-23.

(4) Bjørkøy, G. et al. (2006) Autophagy 2, 138-9.

(5) Joung, I. et al. (1996) Proc Natl Acad Sci USA 93, 5991-5.

(6) Sanchez, P. et al. (1998) Mol Cell Biol 18, 3069-80.

(7) Puls, A. et al. (1997) Proc Natl Acad Sci USA 94, 6191-6.

(8) Vadlamudi, R.K. et al. (1996) J Biol Chem 271, 20235-7.

(9) Wooten, M.W. et al. (2005) J Biol Chem 280, 35625-9.

(10) Bjørkøy, G. et al. (2005) J Cell Biol 171, 603-14.

(11) Komatsu, M. et al. (2007) Cell 131, 1149-63.

(12) Pankiv, S. et al. (2007) J Biol Chem 282, 24131-45.

<u>п</u>

 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
 AII—all species expected
 Species enclosed in parentheses are predicted to react based on 100% homology.