

Store at  
-20°C

#14393

## SignalSilence® OSR1 siRNA I

www.cellsignal.com

Support: 877-678-TECH (8324)  
www.cellsignal.com/supportOrders: 877-616-CELL (2355)  
orders@cellsignal.comEntrez-Gene ID #9943  
UniProt ID #095747

New 08/14

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

**Species Cross-Reactivity: H**

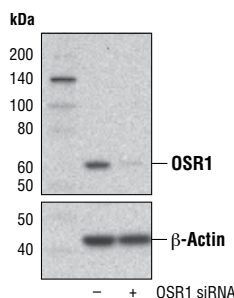
**Description:** SignalSilence® OSR1 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit OSR1 expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

**Background:** SPAK (STE20/SPS1-related Pro/Ala-rich kinase) and OSR1 (oxidative stress responsive 1) are members of the GCK family serine/threonine kinases. Overexpression and *in vitro* studies demonstrate that SPAK is able to activate p38 MAP kinase indicating a possible role for SPAK in the stress response (1). Yeast two-hybrid screening revealed that SPAK and OSR1 bind to Na-K-2Cl cotransporters NKCC1 and NKCC2 and K-Cl cotransporter KCC3 (2). WNK1 and WNK4 phosphorylate SPAK at Thr243/247 and Ser380 (3-5). Similarly, WNK1 and WNK4 phosphorylate OSR1 at Thr185 and Ser315 (3,4). Phosphorylation at these sites stimulates SPAK and OSR1 activity, leading to NKCC1 phosphorylation and enhanced NKCC1 activity (3-5). SPAK is also phosphorylated at Ser311 by PKC $\theta$  in response to T cell activation. Substitution of Ser311 with Ala or specific siRNA knock-down of SPAK dramatically reduces TCR/CD28-induced AP-1 activation, suggesting SPAK is involved in T cell signaling as well (6).

**Directions for Use:** CST recommends transfection with 100 nM SignalSilence® OSR1 siRNA I 48 to 72 hours prior to cell lysis. For transfection procedure, follow the 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  $\mu$ 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® OSR1 siRNA I (+), using OSR1 Antibody #3729 (upper) or  $\beta$ -Actin (D6A8) Rabbit mAb #8457 (lower). The OSR1 Antibody confirms silencing of OSR1 expression, while the  $\beta$ -Actin (D6A8) Rabbit mAb is used as a loading control.

**Storage:** OSR1 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](http://www.cellsignal.com)

**Background References:**

- (1) Johnston, A.M. et al. (2000) *Oncogene* 19, 4290-7.
- (2) Piechotta, K. et al. (2002) *J Biol Chem* 277, 50812-9.
- (3) Vitari, A.C. et al. (2005) *Biochem J* 391, 17-24.
- (4) Moriguchi, T. et al. (2005) *J Biol Chem* 280, 42685-93.
- (5) Gagnon, K.B. et al. (2006) *Mol Cell Biol* 26, 689-98.
- (6) Li, Y. et al. (2004) *EMBO J* 23, 1112-22.

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