

## SignalSilence® SET8 siRNA I



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New 09/13

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

**Species Cross-Reactivity: H, (Mk)**

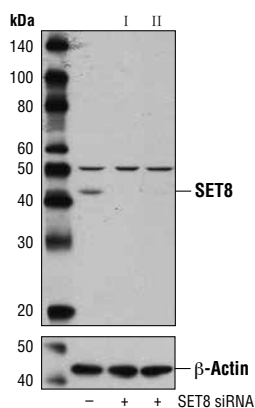
**Description:** SignalSilence® SET8 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit SET8 expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products are rigorously tested in-house and have been shown to reduce protein expression by western analysis.

**Background:** SET domain-containing lysine methyltransferase 8 (SET8), also known as PR/SET domain-containing protein 7 (PR/SET7), is a member of a family of histone lysine methyltransferases, each of which contains a conserved catalytic SET domain originally identified in *Drosophila* Su[var]3-9, Enhancer of zeste, and Trithorax proteins (1-3). SET8 is a single-subunit enzyme that mono-methylates histone H4 on Lys20, preferably on nucleosomal substrates (1-3). SET8 protein levels and Histone H4 Lys20 methylation are cell cycle regulated, both increasing in S phase and peaking at G2/M phase (4,5). SET8 interacts with the PCNA protein, associates with sites of active DNA synthesis, and is required for DNA replication and genome stability during S phase (5-7). Inhibition of SET8 using shRNA or siRNA results in arrest of replication forks, induction of double-stranded DNA breaks, and a Chk1-mediated cell-cycle arrest in S and G2/M phases of the cell cycle (6,7). Furthermore, SET8 methylates p53 on Lys382, down regulating the pro-apoptotic and checkpoint activation functions of p53 (8). In response to DNA damage, SET8 expression levels decrease, allowing p53 to activate checkpoints and/or apoptosis (8). Both the methylation of histone H4 Lys20 and p53 appear to be important for the functions of SET8 in S phase.

**Specificity/Sensitivity:** SignalSilence® SET8 siRNA I will inhibit human and monkey SET8 expression.

**Directions for Use:** CST recommends transfection with 100 nM SET8 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.



Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® SET8 siRNA I (+) or SignalSilence® SET8 siRNA II #13107 (+), using SET8 (C18B7) Rabbit mAb #2996 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The SET8 (C18B7) Rabbit mAb, which cross-reacts with an unidentified protein at 50 kDa, confirms silencing of SET8 expression while the β-Actin (D6A8) Rabbit mAb is used as a loading control.

**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.

Entrez-Gene ID #387893  
Swiss-Prot Acc. #Q9NQR1

**Storage:** SET8 siRNA I is supplied in RNase-free water. Aliquot and store at -20°C.

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**Background References:**

- (1) Fang, J. et al. (2002) *Curr Biol* 12, 1086-99.
- (2) Xiao, B. et al. (2005) *Genes Dev* 19, 1444-54.
- (3) Couture, J.F. et al. (2005) *Genes Dev* 19, 1455-65.
- (4) Rice, J.C. et al. (2002) *Genes Dev* 16, 2225-30.
- (5) Huen, M.S. et al. (2008) *J Biol Chem* 283, 11073-7.
- (6) Tardat, M. et al. (2007) *J Cell Biol* 179, 1413-26.
- (7) Jørgensen, S. et al. (2007) *J Cell Biol* 179, 1337-45.
- (8) Shi, X. et al. (2007) *Mol Cell* 27, 636-46.v