Store at -20°C

# SignalSilence<sup>®</sup> RIP3 siRNA I (Mouse Specific)

### www.cellsignal.com

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> Entrez-Gene ID #56532

UniProt ID #Q9QZL0

New 10/14

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

#### **Species Cross-Reactivity: M**

**Description:** SignalSilence<sup>®</sup> RIP3 siRNA I (Mouse Specific) from Cell Signaling Technology (CST) allows the researcher to specifically inhibit RIP3 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:** The receptor-interacting protein (RIP) family of serine-threonine kinases (RIP, RIP2, RIP3, and RIP4) are important regulators of cellular stress that trigger pro-survival and inflammatory responses through the activation of NF-KB, as well as pro-apoptotic pathways (1). In addition to the kinase domain, RIP contains a death domain responsible for interaction with the death domain receptor Fas and recruitment to TNF-R1 through interaction with TRADD (2,3). RIP-deficient cells show a failure in TNF-mediated NF-kB activation, making the cells more sensitive to apoptosis (4,5). RIP also interacts with TNFreceptor-associated factors (TRAFs) and can recruit IKKs to the TNF-R1 signaling complex via interaction with NEMO, leading to  $I\kappa B$  phosphorylation and degradation (6,7). Overexpression of RIP induces both NF-KB activation and apoptosis (2,3). Caspase-8-dependent cleavage of the RIP death domain can trigger the apoptotic activity of RIP (8).

Receptor-interacting protein 3 (RIP3) was originally found to interact with RIP and the TNF receptor complex to induce apoptosis and activation of NF- $\kappa$ B (9,10). It has subsequently been shown that the association between RIP and RIP3 is a key component of a signaling pathway that results in programmed necrosis (necroptosis), a necrotic-like cell death induced by TNF in the presence of caspase inhibitors (11-13). RIP3 is phosphorylated at Ser227 and targets the phosphorylation of mixed lineage kinase domain-like protein (MLKL), which is critical for necroptosis (14).

**Directions for Use:** CST recommends transfection with 100 nM RIP3 siRNA I (Mouse Specific) 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  $\mu$ 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. kDa I II 200 140 100 80 60 50 RIP3 40 30 20 140 100 a-Actinin 80 + + **RIP3 siRNA** 

Western blot analysis of extracts from L929 cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® RIP3 siRNA I (Mouse Specific) (+), or SignalSilence® RIP3 siRNA II (Mouse Specific) #14661 (+), using RIP3 Antibody (Rodent Specific) #14401 (upper) and  $\alpha$ -Actinin (D6F6) XP® Rabbit mAb #6487 (lower). The RIP3 Antibody (Rodent Specific) confirms silencing of RIP3 expression, while the  $\alpha$ -Actinin (D6F6) XP® Rabbit mAb is used as a loading control.

**Storage:** RIP3 siRNA I (Mouse Specific) is supplied in RNAsefree 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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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