

Store at  
-20°C

#14712

# SignalSilence® RBL2 siRNA I

www.cellsignal.com

10 µM in 300 µl (3 nmol)

Support: 877-678-TECH (8324)  
www.cellsignal.com/supportOrders: 877-616-CELL (2355)  
orders@cellsignal.comEntrez-Gene ID #5934  
UniProt ID #Q08999

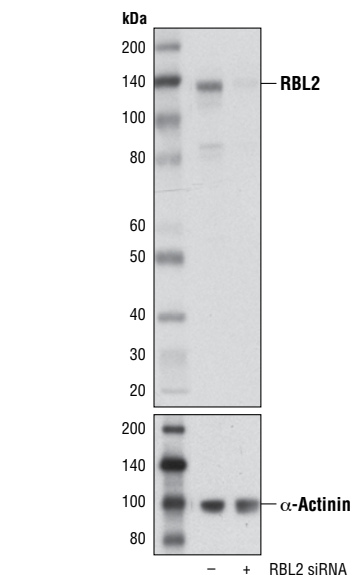
New 12/14

**For Research Use Only. Not For Use In Diagnostic Procedures.****Species Cross-Reactivity: H**

**Description:** SignalSilence® RBL2 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit RBL2 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:** The retinoblastoma (Rb) tumor suppressor family includes the retinoblastoma protein Rb (p105), retinoblastoma-like protein 1 (RBL1, p107), and retinoblastoma-like protein 2 (RBL2, p130). These Rb family proteins are referred to as 'pocket proteins' because they contain a conserved binding pocket region that interacts with critical regulatory proteins, including E2F family transcription factors, c-Abl tyrosine kinase, and proteins containing a conserved LXCXE motif (1,2). In quiescent G<sub>0</sub> phase cells, active Rb proteins are hypophosphorylated and bind to E2F transcription factors to repress transcription and inhibit cell cycle progression (1,2). Upon growth factor induction of quiescent cells, Rb proteins become hyperphosphorylated and inactivated by G1-phase cyclinD-cdk4/6, G1/S-phase cyclin E-cdk2, and G1/S-phase cyclin A-cdk2 complexes (1,2). Hyperphosphorylation of Rb proteins results in a loss of E2F binding and allows for transcriptional activation and cell cycle progression (1,2). In addition to regulating the cell cycle, Rb proteins regulate chromosome stability, induction, and maintenance of senescence, apoptosis, cellular differentiation, and angiogenesis (3).

Retinoblastoma-like protein 2 (RBL2, p130) is the most predominant and active Rb family member found in quiescent cells. In these cells, RBL2 interacts with E2F4 and E2F5 to recruit the DP, RB-like, E2F, and MuvB protein (DREAM) complex to E2F target genes to repress transcription of multiple genes required for progression into S phase and mitosis (4-6). Hypophosphorylation of RBL2 during cellular senescence is required for maintenance of senescent cells (7,8).



Western blot analysis of extracts from 293 cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® RBL2 siRNA I (+), using RBL2 (D9T7M) Rabbit mAb #13610 (upper) and α-Actinin (D6F6) XP® Rabbit mAb #6487 (lower). The RBL2 (D9T7M) Rabbit mAb confirms silencing of RBL2 expression, while the α-Actinin (D6F6) XP® Rabbit mAb is used as a loading control.

**Directions for Use:** CST recommends transfection with 100 nM SignalSilence® RBL2 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.

**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.

**Storage:** RBL2 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:**

- (1) Du, W. and Pogoriler, J. (2006) *Oncogene* 25, 5190-200.
- (2) Giacinti, C. and Giordano, A. (2006) *Oncogene* 25, 5220-7.
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- (4) Moberg, K. et al. (1996) *Mol Cell Biol* 16, 1436-49.
- (5) Takahashi, Y. et al. (2000) *Genes Dev* 14, 804-16.
- (6) Smith, E.J. et al. (1996) *Mol Cell Biol* 16, 6965-76.
- (7) Kapić, A. et al. (2006) *Cell Death Differ* 13, 324-34.
- (8) Helmbold, H. et al. (2009) *Oncogene* 28, 3456-67.

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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.