

#6542 Store at -20°C

SignalSilence® Rb siRNA II



✓ 10 µM in 300 µl (100 Transfections)

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

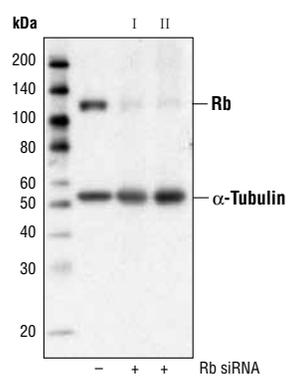
Species Cross-Reactivity: H, M, R

Description: SignalSilence® Rb siRNA II from Cell Signaling Technology (CST) allows the researcher to specifically inhibit Rb 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 target protein expression by western analysis.

Background: The retinoblastoma tumor suppressor protein, Rb, regulates cell proliferation by controlling progression through the restriction point within the G1-phase of the cell cycle (1). Rb has three functionally distinct binding domains and interacts with critical regulatory proteins including the E2F family of transcription factors, c-Abl tyrosine kinase and proteins with a conserved LXCXE motif (2-4). Cell cycle-dependent phosphorylation by a CDK inhibits Rb target binding and allows cell cycle progression (5). Rb inactivation and subsequent cell cycle progression likely requires an initial phosphorylation by cyclin D-CDK4/6 followed by cyclin E-CDK2 phosphorylation (6). Specificity of different CDK/cyclin complexes has been observed *in vitro* (6-8) and cyclin D1 is required for Ser780 phosphorylation *in vivo* (9).

Directions for Use: CST recommends transfection with 100 nM Rb 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.



Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Fluorescein Conjugate) #6201 (-), SignalSilence® Rb siRNA I #6451 (+) or SignalSilence® Rb siRNA II (+), using Rb (4H1) Mouse mAb #9309 and α-Tubulin (11H10) Rabbit mAb #2125. The Rb (4H1) Mouse mAb confirms silencing of Rb expression, while the α-Tubulin (11H10) Rabbit mAb is used to control for loading and specificity of Rb siRNA.

Entrez-Gene ID #5925
Swiss-Prot Acc. #P06400

Storage: Rb 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) Sherr, C.J. (1996) *Science* 274, 1672-7.
- (2) Nevins, J.R. (1992) *Science* 258, 424-9.
- (3) Welch, P.J. and Wang, J.Y. (1993) *Cell* 75, 779-90.
- (4) Hu, Q.J. et al. (1990) *EMBO J* 9, 1147-55.
- (5) Knudsen, E.S. and Wang, J.Y. (1997) *Mol Cell Biol* 17, 5771-83.
- (6) Lundberg, A.S. and Weinberg, R.A. (1998) *Mol Cell Biol* 18, 753-61.
- (7) Connell-Crowley, L. et al. (1997) *Mol Biol Cell* 8, 287-301.
- (8) Kitagawa, M. et al. (1996) *EMBO J* 15, 7060-9.
- (9) Geng, Y. et al. (2001) *Proc Natl Acad Sci USA* 98, 194-9.

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