#13015 Store at -20°C

SignalSilence® Cyclin D1 siRNA II

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

New 08/13

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

Species Cross-Reactivity: H, (Mk)

Description: SignalSilence® Cyclin D1 siRNA II from Cell Signaling Technology (CST) allows the researcher to specifically inhibit cyclin D1 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.

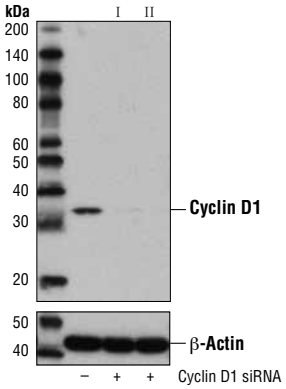
Background: Activity of the cyclin-dependent kinases CDK4 and CDK6 is regulated by T-loop phosphorylation, by the abundance of their cyclin partners (the D-type cyclins), and by association with CDK inhibitors of the Cip/Kip or INK family of proteins (1). The inactive ternary complex of cyclin D/CDK4 and p27 Kip1 requires extracellular mitogenic stimuli for the release and degradation of p27 concomitant with a rise in cyclin D levels to affect progression through the restriction point and Rb-dependent entry into S-phase (2). The active complex of cyclin D/CDK4 targets the retinoblastoma protein for phosphorylation, allowing the release of E2F transcription factors that activate G1/S-phase gene expression (3). Levels of cyclin D protein drop upon withdrawal of growth factors through downregulation of protein expression and phosphorylation-dependent degradation (4).

Specificity/Sensitivity: SignalSilence® Cyclin D1 siRNA II inhibits human and monkey cyclin D1 expression.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® Cyclin D1 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.

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.



Western blot analysis of extracts from MCF7 cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® Cyclin D1 siRNA I #12962 (+), or SignalSilence® Cyclin D1 siRNA II (+), using Cyclin D1 (92G2) Rabbit mAb #2978 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The Cyclin D1 (92G2) Rabbit mAb confirms silencing of cyclin D1 expression, while the β-Actin (D6A8) Rabbit mAb is used as a loading control.

Entrez-Gene ID #595
UniProt Acc. #P24385

Storage: Cyclin D1 siRNA II is supplied in RNAse-free water. *Aliquot and store at -20°C.*

For product specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Hirai, H. et al. (1995) *Mol. Cell. Biol.* 15, 2672-2681.
(2) Sherr, C.J. (1996) *Science* 274, 1672-1677.
(3) Lukas, J. et al. (1996) *Mol. Cell. Biol.* 16, 6917-6925.
(4) Diehl, J.A. et al. (1997) *Genes Dev.* 11, 957-972.

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.