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# cyclin D1 shRNA (h2) Lentiviral Particles: sc-44257-V

## BACKGROUND

The proliferation of eukaryotic cells is controlled at specific points in the cell cycle, particularly at the G<sub>1</sub>/S and the G<sub>2</sub>/M transitions. It is well established that the Cdc2 p34-cyclin B protein kinase plays a critical role in the G<sub>2</sub>/M transition while cyclin A associates with Cdk2 p33 and functions in S phase. Considerable effort directed towards the identification of G<sub>1</sub> cyclins has led to the isolation of cyclin D, cyclin C and cyclin E. Of these, cyclin D corresponds to a putative human oncogene, designated PRAD1, which maps at the site of the Bcl1 rearrangement in certain lymphomas and leukemias. Two additional human type D cyclins, as well as their mouse homologs, have been identified. Evidence has established that members of the cyclin D family function to regulate phosphorylation of the retinoblastoma gene product, thereby activating E2F transcription factors.

## REFERENCES

1. Draetta, G. 1990. Cell cycle control in eukaryotes: molecular mechanisms of Cdc2 activation. *Trends Biol. Sci.* 15: 378-383.
2. Xiong, Y., et al. 1991. Human D-type cyclin. *Cell* 65: 691-699.
3. Kiyokawa, H., et al. 1992. Cloning of a D-type cyclin from murine erythroleukemia cells. *Proc. Natl. Acad. Sci. USA* 89: 2444-2447.
4. Won, K., et al. 1992. Growth-regulated expression of D-type cyclin genes in human diploid fibroblasts. *Proc. Natl. Acad. Sci. USA* 89: 9910-9914.
5. Motokura, T., et al. 1992. Cloning and characterization of human cyclin D3, a cDNA closely related in sequence to the PRAD1/cyclin D1 proto-oncogene. *J. Biol. Chem.* 267: 20412-20415.
6. Inaba, T., et al. 1992. Genomic organization, chromosomal localization, and independent expression of human cyclin D genes. *Genomics* 13: 565-574.

## CHROMOSOMAL LOCATION

Genetic locus: CCND1 (human) mapping to 11q13.3.

## PRODUCT

cyclin D1 shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10<sup>6</sup> infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see cyclin D1 siRNA (h2): sc-44257 and cyclin D1 shRNA Plasmid (h2): sc-44257-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

cyclin D1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of cyclin D1 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10<sup>6</sup> infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

cyclin D1 (H-295): sc-753 is recommended as a control antibody for monitoring of cyclin D1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor cyclin D1 gene expression knockdown using RT-PCR Primer: cyclin D1 (h2)-PR: sc-44257-PR (20 µl, 565 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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