

cyclin D1 siRNA (h2): sc-44257

BACKGROUND

The proliferation of eukaryotic cells is controlled at specific points in the cell cycle, particularly at the G₁/S and the G₂/M transitions. It is well established that the Cdc2 p34-cyclin B protein kinase plays a critical role in the G₂/M transition while cyclin A associates with Cdk2 p33 and functions in S phase. Considerable effort directed towards the identification of G₁ 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.

CHROMOSOMAL LOCATION

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

PRODUCT

cyclin D1 siRNA (h2) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see cyclin D1 shRNA Plasmid (h2): sc-44257-SH and cyclin D1 shRNA (h2) Lentiviral Particles: sc-44257-V as alternate gene silencing products.

For independent verification of cyclin D1 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44257A, sc-44257B and sc-44257C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

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

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

cyclin D1 (A-12): sc-8396 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

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 μ l, 565 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Gravina, G.L., et al. 2014. Torc1/Torc2 inhibitor, Palomid 529, enhances radiation response modulating CRM1-mediated survivin function and delaying DNA repair in prostate cancer models. *Prostate* 74: 852-868.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.