

Cdk4 siRNA (m): sc-29262

BACKGROUND

Cell cycle progression is controlled in part by a family of cyclin proteins and cyclin dependent kinases (Cdks). Cdk proteins work in concert with the cyclins to phosphorylate key substrates involved in each phase of cell cycle progression. Another family of proteins, Cdk inhibitors, also plays a role in regulating the cell cycle by binding to cyclin-Cdk complexes and modulating their activity. Several Cdk proteins have been identified, including Cdk2-Cdk8, PCTAIRE-1-3, PITSLRE and PITSLRE. Cdk4, in complex with D-type cyclins, is thought to regulate cell growth during the G₁ phase of the cell cycle. This association with a D-type cyclin upregulates Cdk4 activity, whereas binding to the Cdk inhibitor p16 downregulates Cdk4 activity. Activation of the Cdk4-cyclin complexes requires phosphorylation on a single threonyl residue of Cdk4, catalyzed by a Cdk-activating protein (CAK).

REFERENCES

- Okuda, T., et al. 1992. PCTAIRE-1 and PCTAIRE-2: two members of a novel Cdc2/Cdc28-related protein kinase gene family. *Oncogene* 7: 2249-2258.
- Serrano, M., et al. 1993. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/Cdk4. *Nature* 366: 704-707.
- Pines, J. 1994. The cell cycle kinases. *Semin. Cancer Biol.* 5: 305-313.
- Kato, J.Y., et al. 1994. Regulation of cyclin D-dependent kinase (Cdk4) by Cdk4-activating kinase. *Mol. Cell. Biol.* 14: 2713-2721.
- Matsuoka, M., et al. 1994. Activation of cyclin-dependent kinase 4 (Cdk4) by mouse M015-associated kinase. *Mol. Cell. Biol.* 14: 7265-7275.

CHROMOSOMAL LOCATION

Genetic locus: Cdk4 (mouse) mapping to 10 D3.

PRODUCT

Cdk4 siRNA (m) 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 Cdk4 shRNA Plasmid (m): sc-29262-SH and Cdk4 shRNA (m) Lentiviral Particles: sc-29262-V as alternate gene silencing products.

For independent verification of Cdk4 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29262A, sc-29262B and sc-29262C.

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

Cdk4 siRNA (m) is recommended for the inhibition of Cdk4 expression in mouse 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

Cdk4 (DCS-35): sc-23896 is recommended as a control antibody for monitoring of Cdk4 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 Cdk4 gene expression knockdown using RT-PCR Primer: Cdk4 (m)-PR: sc-29262-PR (20 μ l, 593 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Hashimoto, K., et al. 2006. C/EBP α inactivation in FAK-overexpressed HL-60 cells impairs cell differentiation. *Cell. Signal.* 18: 955-963.
- Schmetsdorf, S., et al. 2009. A putative role for cell cycle-related proteins in microtubule-based neuroplasticity. *Eur. J. Neurosci.* 29: 1096-1107.
- Adon, A.M., et al. 2010. Cdk2 and Cdk4 regulate the centrosome cycle and are critical mediators of centrosome amplification in p53-null cells. *Mol. Cell. Biol.* 30: 694-710.
- Lee, S.H., et al. 2021. CDK4 has the ability to regulate Aurora B and Cenpp expression in mouse keratinocytes. *Oncol. Lett.* 22: 732.

RESEARCH USE

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