



Cdk6 siRNA (m): sc-35048

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–PCTAIRE-3, PITALRE and PITSIRE. Cdk6 is known to associate with cyclins D1, D2 and D3 and to be involved with the G₁/S transition of the cell cycle. Multiple inhibitors of Cdk6 have been identified, including p18 and p19. These inhibitors bind to both free and complexed Cdk6, and they inhibit the activity of the cyclin D-bound Cdk6.

REFERENCES

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- Lucas, J.J., et al. 1995. Regulation of synthesis and activity of the PLSTIRE protein (cyclin-dependent kinase 6 (Cdk6)), a major cyclin D-associated Cdk4 homologue in normal human T lymphocytes. *J. Immunol.* 154: 6275-6284.
- Hirai, H., et al. 1995. Novel INK4 proteins, p19 and p18, are specific inhibitors of the cyclin D-dependent kinases Cdk4 and Cdk6. *Mol. Cell. Biol.* 15: 2672-2681.
- Chan, F.K., et al. 1995. Identification of human and mouse p19, a novel Cdk4 and Cdk6 inhibitor with homology to p16^{INK4}. *Mol. Cell. Biol.* 15: 2682-2688.
- Siebert, R., et al. 1996. Role of the cyclin-dependent kinase 4 and 6 inhibitor gene family p15, p16, p18 and p19 in leukemia and lymphoma. *Leuk. Lymphoma* 23: 505-520.

CHROMOSOMAL LOCATION

Genetic locus: Cdk6 (mouse) mapping to 5 A1.

PRODUCT

Cdk6 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 Cdk6 shRNA Plasmid (m): sc-35048-SH and Cdk6 shRNA (m) Lentiviral Particles: sc-35048-V as alternate gene silencing products.

For independent verification of Cdk6 (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-35048A, sc-35048B and sc-35048C.

RESEARCH USE

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

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

Cdk6 siRNA (m) is recommended for the inhibition of Cdk6 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

Cdk6 (B-10): sc-7961 is recommended as a control antibody for monitoring of Cdk6 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 Cdk6 gene expression knockdown using RT-PCR Primer: Cdk6 (m)-PR: sc-35048-PR (20 μ l, 551 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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