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Cdk7 siRNA (h2): sc-43678

BACKGROUND

Progression through the cell cycle requires activation of a series of enzymes designated cyclin dependent kinases (Cdks). The monomeric catalytic subunit Cdk2, a critical enzyme for initiation of cell cycle progression, is completely inactive. Partial activation is achieved by the binding of regulatory cyclins such as cyclin D1, while full activation requires additional phosphorylation at Thr 160. The enzyme responsible for the phosphorylation of Cdk2 on Thr 160 and also of Cdc2 p34 on Thr 161, designated Cdk-activating kinase (CAK), has been partially purified and shown to be comprised of a catalytic subunit and a regulatory subunit. The catalytic subunit, designated Cdk7, has been identified as the mammalian homolog of MO15, a protein kinase demonstrated in starfish and *Xenopus*. The regulatory subunit is a novel cyclin (cyclin H) and is required for activation of Cdk7. Like other Cdks, Cdk7 contains a conserved threonine residue required for full activity; mutation of this residue severely reduces CAK activity.

REFERENCES

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- Kato, J.Y., et al. 1994. Regulation of cyclin D-dependent kinase 4 (Cdk4) by Cdk4-activating kinase. *Mol. Cell. Biol.* 14: 2713-2721.
- Lavedakou, E.N., et al. 1994. Two novel human serine/threonine kinases with homologies to the cell cycle regulating *Xenopus* MO15, and NIMA kinases: cloning and characterization of their expression pattern. *Oncogene* 9: 1977-1988.
- Matsuoka, M., et al. 1994. Activation of cyclin-dependent kinase 4 (Cdk4) by mouse MO15-associated kinase. *Mol. Cell. Biol.* 14: 7265-7275.
- Pinhero, R., et al. 2004. A uniform procedure for the purification of CDK7/CycH/MAT1, CDK8/CycC and CDK9/CycT1. *Biol. Proced. Online* 6: 163-172.
- Lolli, G., et al. 2004. The crystal structure of human CDK7 and its protein recognition properties. *Structure* 12: 2067-2079.

CHROMOSOMAL LOCATION

Genetic locus: CDK7 (human) mapping to 5q13.2.

PRODUCT

Cdk7 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 Cdk7 shRNA Plasmid (h2): sc-43678-SH and Cdk7 shRNA (h2) Lentiviral Particles: sc-43678-V as alternate gene silencing products.

For independent verification of Cdk7 (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-43678A, sc-43678B and sc-43678C.

PROTOCOLS

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

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

Cdk7 siRNA (h2) is recommended for the inhibition of Cdk7 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

Cdk7 (C-4): sc-7344 is recommended as a control antibody for monitoring of Cdk7 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 Cdk7 gene expression knockdown using RT-PCR Primer: Cdk7 (h2)-PR: sc-43678-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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