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# Cdk7 shRNA (h2) Lentiviral Particles: sc-43678-V

## 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

- Hunter, T., et al. 1994. Cyclins and cancer II: cyclin D and CDK inhibitors come of age. *Cell* 79: 573-582.
- Kato, J.Y., et al. 1994. Regulation of cyclin D-dependent kinase 4 (cdk4) by Cdk4-activating kinase. *Mol. Cell. Biol.* 14: 2713-2721.
- Levedakou, 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 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 Cdk7 siRNA (h2): sc-43678 and Cdk7 shRNA Plasmid (h2): sc-43678-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

Cdk7 shRNA (h2) Lentiviral Particles is recommended for the inhibition of Cdk7 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

Cdk7 (C-5): sc-365075 is recommended as a control antibody for monitoring of 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## 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  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  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.

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