SANTA CRUZ BIOTECHNOLOGY, INC.

cyclin H siRNA (h): sc-29290

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 additionally requires phosphorylation at Thr-160. The enzyme responsible for phosphorylation of Thr-160 of Cdk2 and also Thr-161 in Cdc2 p34, 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 earlier 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 required for full activity; mutation of this residue severely reduces CAK activity.

REFERENCES

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3. King, R.W., et al. 1994. Mitosis in transition. Cell 79: 563-571.
4. Hunter, T., et al. 1994. Cyclins and cancer II: cyclin D and CDK inhibitors come of age. Cell 79: 573-582.
5. Kato, J.Y., et al. 1994. Regulation of cyclin D-dependent kinase 4 (cdk4) by cdk4-activating kinase. Mol. Cell. Biol. 14: 2713-2721.
6. 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.
7. Wu, L., et al. 1994. Molecular cloning of the human CAK1 gene encoding a cyclin-dependent kinase-activating kinase. Oncogene 9: 2089-2096.
8. Matsuoka, M., et al. 1994. Activation of cyclin-dependent kinase 4 (cdk4) by mouse MO15-associated kinase. Mol. Cell. Biol. 14: 7265-7275.

CHROMOSOMAL LOCATION

Genetic locus: CCNH (human) mapping to 5q14.3.

PRODUCT

cyclin H siRNA (h) is a pool of 4 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see cyclin H shRNA Plasmid (h): sc-29290-SH and cyclin H shRNA (h) Lentiviral Particles: sc-29290-V as alternate gene silencing products.

For independent verification of cyclin H (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29290A, sc-29290B, sc-29290C and sc-29290D.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

cyclin H siRNA (h) is recommended for the inhibition of cyclin H 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 µM in 66 µl. 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 H (D-10): sc-1662 is recommended as a control antibody for monitoring of cyclin H 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 H gene expression knockdown using RT-PCR Primer: cyclin H (h)-PR: sc-29290-PR (20 µl, 443 bp). 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.

PROTOCOLS

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