SANTA CRUZ BIOTECHNOLOGY, INC.

Chk1 siRNA (h2): sc-44255

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The Power to Question

BACKGROUND

Cell cycle events are regulated by the sequential activation and deactivation of cyclin dependent kinases (Cdks) and by proteolysis of cyclins. Chk1 and Chk2 are involved in these processes as regulators of Cdks. Chk1 and Chk2 both function as essential components in the G_2 DNA damage checkpoint by phosphorylating Cdc25C in response to DNA damage. Phosphorylation inhibits Cdc25C activity, thereby blocking mitosis. Cdc25A, Cdc25B and Cdc25C protein tyrosine phosphatases function as mitotic activators by dephosphorylating Cdc2 p34 on regulatory tyrosine residues. It has also been shown that Chk1 can phosphorylate Wee 1 *in vitro*, providing evidence that the hyperphosphorylated form of Wee 1, seen in cells delayed by Chk1 overexpression, is due to phosphorylation by Chk1.

REFERENCES

1. Gautier, J., et al. 1991. Cdc25 is a specific tyrosine phosphatase that directly activates $p34^{Cdc2}$. Cell 67: 197-211.
2. Barinaga, M. 1995. A new twist to the cell cycle. Science 269: 631-632.
3. Sanchez, Y., et al. 1997. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science 277: 1497-1501.
4. O'Connell, M.J., et al. 1997. Chk1 is a Wee 1 kinase in the G_2 DNA damage checkpoint inhibiting Cdc2 by Y15 phosphorylation. EMBO J. 16: 545-554.
5. Peng, C.Y., et al. 1997. Mitotic and G_2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on Serine 216. Science 277: 1501-1505.
6. Matsuoka, S., et al. 1998. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science 282: 1893-1897.
7. Collis, S.J., et al. 2007. HCLK2 is essential for the mammalian S-phase checkpoint and impacts on Chk1 stability. Nat. Cell Biol. 9: 391-401.
8. Zachos, G., et al. 2007. Exercising restraints: role of Chk1 in regulating the onset and progression of unperturbed mitosis in vertebrate cells. Cell Cycle 6: 810-813.

CHROMOSOMAL LOCATION

Genetic locus: CHEK1 (human) mapping to 11q24.2.

PRODUCT

Chk1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Chk1 shRNA Plasmid (h2): sc-44255-SH and Chk1 shRNA (h2) Lentiviral Particles: sc-44255-V as alternate gene silencing products.

For independent verification of Chk1 (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-44255A, sc-44255B and sc-44255C.

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

Chk1 siRNA (h2) is recommended for the inhibition of Chk1 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

Chk1 (G-4): sc-8408 is recommended as a control antibody for monitoring of Chk1 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 Chk1 gene expression knockdown using RT-PCR Primer: Chk1 (h2)-PR: sc-44255-PR (20 µl, 425 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.