

Chk2 siRNA (m): sc-29272

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 G₂ 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 Wee1 *in vitro*, providing evidence that the hyperphosphorylated form of Wee1, seen in cells delayed by Chk1 overexpression, is due to phosphorylation by Chk1.

REFERENCES

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2. Barinaga, M. 1995. A new twist to the cell cycle. *Science* 269: 631-632.
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4. 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.
5. Peng, C.Y., et al. 1997. Mitotic and G₂ 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. Zaugg, K., et al. 2007. Cross-talk between Chk1 and Chk2 in double-mutant thymocytes. *Proc. Natl. Acad. Sci. USA* 104: 3805-3810.
8. Abdelmohsen, K., et al. 2007. Phosphorylation of HuR by Chk2 regulates SIRT1 expression. *Mol. Cell* 25: 543-557.

CHROMOSOMAL LOCATION

Genetic locus: Chk2 (mouse) mapping to 5 F.

PRODUCT

Chk2 siRNA (m) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Chk2 shRNA Plasmid (m): sc-29272-SH and Chk2 shRNA (m) Lentiviral Particles: sc-29272-V as alternate gene silencing products.

For independent verification of Chk2 (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-29272A, sc-29272B, sc-29272C and sc-29272D.

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

Chk2 siRNA (m) is recommended for the inhibition of Chk2 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

Chk2 (A-11): sc-17747 is recommended as a control antibody for monitoring of Chk2 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 Chk2 gene expression knockdown using RT-PCR Primer: Chk2 (m)-PR: sc-29272-PR (20 μ l, 456 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.