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Chk1 shRNA (h2) Lentiviral Particles: sc-44255-V

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 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

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- Barinaga, M. 1995. A new twist to the cell cycle. *Science* 269: 631-632.
- 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.
- O'Connell, M.J., et al. 1997. Chk1 is a Wee 1 kinase in the G₂ DNA damage checkpoint inhibiting Cdc2 by Y15 phosphorylation. *EMBO J.* 16: 545-554.
- 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.
- Matsuoka, S., et al. 1998. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science* 282: 1893-1897.

CHROMOSOMAL LOCATION

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

PRODUCT

Chk1 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 µl frozen stock containing 1.0 x 10⁶ 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 Chk1 siRNA (h2): sc-44255 and Chk1 shRNA Plasmid (h2): sc-44255-SH as alternate gene silencing products.

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.

APPLICATIONS

Chk1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of Chk1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

Chk1 (D-7): sc-377231 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 (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 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.

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.

STORAGE

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

PROTOCOLS

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