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Plk shRNA (h2) Lentiviral Particles: sc-44322-V

BACKGROUND

Plk (for polo-like kinase) encodes a serine/threonine kinase that is closely related to polo and CDC5, genes that are required for passage through mitosis in *Drosophila* and *Saccharomyces*, respectively. Polo and CDC5 both code for proteins that are involved in regulating the function of the mitotic spindle. Plk protein accumulates in the cell during the S and G₂ phases of the cell cycle and both protein content and catalytic activity peak at the onset of mitosis, followed by a rapid reduction after mitosis. Plk expression is detectable in mitotically active tissues such as colon and placenta, as well as in tumors of various origins. It has also been suggested that Plk may serve as a marker of cell proliferation.

REFERENCES

1. Sunkel, C.E. and Glover, D.M. 1988. Polo, a mitotic mutant of *Drosophila* displaying abnormal spindle poles. *J. Cell Sci.* 89: 25-38.
2. Kitada, K., et al. 1993. A multicopy suppressor gene of the *Saccharomyces cerevisiae* G₁ cell cycle mutant gene DBF4 encodes a protein kinase and is identified as CDC5. *Mol. Cell. Biol.* 13: 4445-4457.
3. Lake, R.J. and Jelinek, W.R. 1993. Cell cycle- and terminal differentiation-associated regulation of the mouse mRNA encoding a conserved mitotic protein kinase. *Mol. Cell. Biol.* 13: 7793-7801.
4. Hamanaka, R., et al. 1994. Cloning and characterization of human and murine homologues of the *Drosophila* polo serine-threonine kinase. *Cell Growth Differ.* 5: 249-257.
5. Holtrich, U., et al. 1994. Induction and downregulation of Plk, a human serine/threonine kinase expressed in proliferating cells and tumors. *Proc. Natl. Acad. Sci. USA* 91: 1736-1740.
6. Golsteyn, R.M., et al. 1994. Cell cycle analysis and chromosomal localization of human Plk1, a putative homologue of the mitotic kinases *Drosophila* polo and *Saccharomyces cerevisiae* CDC5. *J. Cell Sci.* 107: 1509-1517.

CHROMOSOMAL LOCATION

Genetic locus: PLK1 (human) mapping to 16p12.2.

PRODUCT

Plk 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 Plk siRNA (h2): sc-44322 and Plk shRNA Plasmid (h2): sc-44322-SH as alternate gene silencing products.

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.

APPLICATIONS

Plk shRNA (h2) Lentiviral Particles is recommended for the inhibition of Plk 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

Plk (F-8): sc-17783 is recommended as a control antibody for monitoring of Plk 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 Plk gene expression knockdown using RT-PCR Primer: Plk (h2)-PR: sc-44322-PR (20 µl, 432 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.

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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