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PRC1 shRNA (m) Lentiviral Particles: sc-44346-V

BACKGROUND

Sequential activation and inactivation of Cdk/cyclin complexes regulates the cell cycle. PRC1 (for protein regulating cytokinesis 1) has been identified as a substrate for several Cdks, including Cdc2 and Cdk2. PRC1 binds to the mid-zone of mitotic spindles during anaphase and is localized to the cell midbody during cytokinesis. Depletion of PRC1 has been shown to prevent cellular cleavage, but it has no effect on nuclear division, demonstrating the importance of PRC1 in mitosis. The yeast homolog of PRC1, Ase1, is essential for spindle assembly, elongation and disassembly during mitosis. Ase1 has been shown to undergo degradation mediated by the APC (anaphase-promoting complex) upon entry into G₁ phase.

REFERENCES

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2. King, R.W., et al. 1994. Mitosis in transition. *Cell* 79: 563-571.
3. Sherr, C.J. 1994. G₁ phase progression: cycling on cue. *Cell* 79: 551-555.
4. Pellman, D., et al. 1995. Two microtubule-associated proteins required for anaphase spindle movement in *Saccharomyces cerevisiae*. *J. Cell Biol.* 130: 1373-85.
5. Juang, Y.L., et al. 1997. APC-mediated proteolysis of Ase1 and the morphogenesis of the mitotic spindle. *Science* 275: 1311-1314.
6. Mollinari, C., et al. 2002. PRC1 is a microtubule binding and bundling protein essential to maintain the mitotic spindle midzone. *J. Cell Biol.* 157: 1175-1186.
7. Vogel, T., et al. 2006. Differential expression of polycomb repression complex 1 (PRC1) members in the developing mouse brain reveals multiple complexes. *Dev. Dyn.* 235: 2574-2585.

CHROMOSOMAL LOCATION

Genetic locus: Prc1 (mouse) mapping to 7 D3.

PRODUCT

PRC1 shRNA (m) 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 μ l frozen stock containing 1.0×10^6 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 PRC1 siRNA (m): sc-44346 and PRC1 shRNA Plasmid (m): sc-44346-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.

PROTOCOLS

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

APPLICATIONS

PRC1 shRNA (m) Lentiviral Particles is recommended for the inhibition of PRC1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

PRC1 (C-1): sc-376983 is recommended as a control antibody for monitoring of PRC1 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 PRC1 gene expression knockdown using RT-PCR Primer: PRC1 (m)-PR: sc-44346-PR (20 μ l). 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.