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# p27 shRNA (h2) Lentiviral Particles: sc-44215-V

## BACKGROUND

Cell cycle progression is regulated by a series of cyclin-dependent kinases consisting of catalytic subunits, designated Cdk, as well as activating subunits, designated cyclins. Orderly progression through the cell cycle requires the activation and inactivation of different cyclin-Cdks at appropriate times. A series of proteins has recently been described that function as "mitotic inhibitors". These include p21, the levels of which are elevated upon DNA damage in G<sub>1</sub> in a p53-dependent manner; p16; and a more recently described p16-related inhibitor designated p15. A p21-related protein, p27, has been described as a negative regulator of G<sub>1</sub> progression and speculated to function as a possible mediator of TGFβ-induced G<sub>1</sub> arrest. p27 interacts strongly with D-type cyclins and Cdk4 *in vitro* and, to a lesser extent, with cyclin E and Cdk2.

## REFERENCES

1. Sherr, C.J. 1993. Mammalian G<sub>1</sub> cyclins. *Cell* 73: 1059-1065.
2. El-Deiry, W.S., et al. 1993. WAF1, a potential mediator of p53 tumor suppression. *Cell* 75: 817-825.
3. Xiong, Y., et al. 1993. p21 is a universal inhibitor of cyclin kinases. *Nature* 366: 701-704.
4. Serrano, M., et al. 1993. A new regulatory motif in cell cycle control causing specific inhibition of cyclin D/Cdk4. *Nature* 366: 704-707.
5. Hannon, G.J., et al. 1994. p15INK4B is a potential effector of TGFβ-induced cell cycle arrest. *Nature* 371: 257-260.
6. Polyak, K., et al. 1994. p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor β and contact inhibition to cell cycle arrest. *Genes Dev.* 8: 9-22.
7. Polyak, K., et al. 1994. Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimutagenic signals. *Cell* 78: 59-66.

## CHROMOSOMAL LOCATION

Genetic locus: CDKN1B (human) mapping to 12p13.1.

## PRODUCT

p27 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 10<sup>6</sup> 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 p27 siRNA (h2): sc-44215 and p27 shRNA Plasmid (h2): sc-44215-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

p27 shRNA (h2) Lentiviral Particles is recommended for the inhibition of p27 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μl frozen viral stock containing 1.0 x 10<sup>6</sup> 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

p27 (A-10): sc-393380 is recommended as a control antibody for monitoring of p27 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 p27 gene expression knockdown using RT-PCR Primer: p27 (h2)-PR: sc-44215-PR (20 μl, 482 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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