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p16/p19 ARF shRNA (m) Lentiviral Particles: sc-43988-V

BACKGROUND

The progression of cells through the cell cycle is regulated by a family of proteins designated cyclin-dependent kinases (Cdks). Sequential activation of individual members of this family and their consequent phosphorylation of critical substrates promote orderly progression through the cell cycle. The protein p16INK4A, identified as a negative regulator of the cell cycle, has been shown to bind to and inhibit the activity of the Cdk4/cyclin D complex. p19 ARF, which is unrelated to p16, arises from transcription of an alternative reading frame of the p16 gene. Like p16, p19 ARF has been shown to induce cell cycle arrest. Mice lacking p19 ARF but expressing functional p16 have been shown to develop tumors early in life. Further studies have indicated that p19 ARF may be disrupted in a large percentage of human T cell acute lymphoblastic leukemias.

REFERENCES

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- Serrano, M., et al. 1993. A new regulatory motif in cell cycle control causing specific inhibition of cyclin D/Cdk4. *Nature* 366: 704-707.
- Kamb, A., et al. 1994. A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 264: 436-440.
- Mao, L., et al. 1995. A novel p16INK4A transcript. *Cancer Res.* 55: 2995-2997.
- Quelle, D.E., et al. 1995. Alternative reading frames of the INK4A tumor suppressor gene encode two unrelated proteins capable of inducing cell cycle arrest. *Cell* 83: 993-1000.
- Kamijo, T., et al. 1997. Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell* 91: 649-659.

CHROMOSOMAL LOCATION

Genetic locus: Cdkn2a (mouse) mapping to 4 C4.

PRODUCT

p16/p19 ARF 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 p16/p19 ARF siRNA (m): sc-43988 and p16/p19 ARF shRNA Plasmid (m): sc-43988-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

p16/p19 ARF shRNA (m) Lentiviral Particles is recommended for the inhibition of p16/p19 ARF 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

p19 ARF (M-60): sc-22784 is recommended as a control antibody for monitoring of p16/p19 ARF 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor p16/p19 ARF gene expression knockdown using RT-PCR Primer: p16/p19 ARF (m)-PR: sc-43988-PR (20 μ l, 446 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.