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p21 shRNA (m2) Lentiviral Particles: sc-44271-V

BACKGROUND

It is now well established that cyclins play a positive role in promoting cell cycle transitions via their ability to associate with and activate their cognate cyclin-dependent kinases (Cdks). Cdk2 associates with cyclins A, D and E, and has been implicated in the control of the G₁ to S phase transition in mammals. A novel Cdk-interacting protein, designated p21, Cip1 or WAF1, has been identified in cyclin A, cyclin D1, cyclin E and Cdk2 immunoprecipitates. p21 is a potent, tight-binding inhibitor of Cdks and can inhibit the phosphorylation of Rb by cyclin A-Cdk2, cyclin E-Cdk2, cyclin D1-Cdk4 and cyclin D2-Cdk4 complexes. Expression of p21 is inducible by wildtype, but not mutant, p53. The mouse homolog of p21 is designated CAP20.

REFERENCES

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3. El-Deiry, W.S., et al. 1993. WAF1, a potential mediator of p53 tumor suppression. *Cell* 75: 817-825.
4. Hunter, T. 1993. Braking the cycle. *Cell* 75: 839-841.
5. Kato, J., et al. 1993. Direct binding of cyclin D to the retinoblastoma gene product and pRb phosphorylation by the cyclin D-dependent kinase Cdk4. *Genes Dev.* 7: 331-342.
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7. Gu, Y., et al. 1993. Inhibition of Cdk2 activity *in vivo* by an associated 20 kDa regulatory subunit. *Nature* 366: 707-710.
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CHROMOSOMAL LOCATION

Genetic locus: Cdkn1a (mouse) mapping to 17 A3.3.

PRODUCT

p21 shRNA (m2) 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 p21 siRNA (m2): sc-44271 and p21 shRNA Plasmid (m2): sc-44271-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

p21 shRNA (m2) Lentiviral Particles is recommended for the inhibition of p21 expression in mouse 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

p21 (F-8): sc-271610 is recommended as a control antibody for monitoring of p21 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 p21 gene expression knockdown using RT-PCR Primer: p21 (m2)-PR: sc-44271-PR (20 µl, 600 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.