SANTA CRUZ BIOTECHNOLOGY, INC.

p19 INK4D siRNA (m): sc-36147

BACKGROUND

The normal progression of cells through the cell cycle is under the control of the cyclin dependent protein kinases Cdk4 and Cdk6, which are subject to inhibition by the mitotic inhibitory protein, p16 INK4A. Isolated members of the p16 INK4A family have been designated p15 INK4B, p18 INK4C and p19 INK4D. p15 INK4B expression is upregulated approximately 30-fold in TGFβ-treated human keratinocytes, suggesting that p15 INK4B may function as an effector of TGFβ-mediated cell cycle arrest through inhibition of Cdk4 and Cdk6 kinases. The gene encoding p15 INK4B has been mapped to chromosome 9p21.3 at a position adjacent to the p16 INK4A gene, at a site of frequent chromosomal abnormality in human tumors. Two p16 INK4A-related proteins, p19 INK4D and p18 INK4C, specifically inhibit the kinase activities of Cdk4 and Cdk6 but do not affect those of cyclin E-Cdk2, cyclin A-Cdk2 or cyclin B-Cdc2 complexes. p19 INK4D is expressed at maximal level during S phase, while overexpression of p19 INK4D leads to G_1 arrest.

REFERENCES

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2. Kamb, A., et al. 1994. A cell cycle regulator potentially involved in genesis of many tumor types. Science 264: 436-440.
3. Hannon, G.J., et al. 1994. p15 INK4B is a potential effector of TGFβ-induced cell cycle arrest. Nature 371: 257-261.
4. Guan, K.L., et al. 1994. Growth suppression by p18, a p16 INK4/MTS1 and p14 INK4B/MTS2-related Cdk6 inhibitor, correlates with wild-type pRb function. Genes Dev. 8: 2939-2952.
5. Hussussian, C.J., et al. 1994. Germline p16 mutations in familial melanoma. Nat. Genet. 8: 15.
6. Cairns, P., et al. 1994. Rates of p16 MTS1 mutations in primary tumors with 9p loss. Science 265: 415-417.
7. Hirai, H., et al. 1995. Novel INK4 proteins, p19 and p18, are specific inhibitors of the Cyclin D-dependent kinases CDK4 and CDK6. Mol. Cell. Biol. 15: 2672-2681.

CHROMOSOMAL LOCATION

Genetic locus: Cdkn2d (mouse) mapping to 9 A3.

PRODUCT

p19 INK4D siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see p19 INK4D shRNA Plasmid (m): sc-36147-SH and p19 INK4D shRNA (m) Lentiviral Particles: sc-36147-V as alternate gene silencing products.

For independent verification of p19 INK4D (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-36147A, sc-36147B and sc-36147C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

p19 INK4D siRNA (m) is recommended for the inhibition of p19 INK4D expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

p19 INK4D (E-11): sc-1665 is recommended as a control antibody for monitoring of p19 INK4D 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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor p19 INK4D gene expression knockdown using RT-PCR Primer: p19 INK4D (m)-PR: sc-36147-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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