SANTA CRUZ BIOTECHNOLOGY, INC.

p16 INK4A/p19 ARF siRNA (m): sc-43988

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 p16 INK4A, 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 INK4A, arises from transcription of an alternative reading frame of the p16 gene. Like p16 INK4A, p19 ARF has been shown to induce cell cycle arrest. Mice lacking p19 ARF but expressing functional p16 INK4A 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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2. Hunter, T. 1993. Braking the cycle. Cell 75: 839-841.
3. Serrano, M., et al. 1993. A new regulatory motif in cell cycle control causing specific inhibition of cyclin D/Cdk4. Nature 366: 704-707.
4. Kamb, A., et al. 1994. A cell cycle regulator potentially involved in genesis of many tumor types. Science 264: 436-440.
5. Mao, L., et al. 1995. A novel p16 INK4A transcript. Cancer Res. 55: 2995-2997.
6. 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.
7. Kamijo, T., et al. 1997. Tumor suppression at the mouse INK4A locus mediated by the alternative reading frame product p19 ARF. Cell 91: 649-659.
8. Gardie, B., et al. 1998. Genomic alterations of the p19 ARF encoding exons in T cell acute lymphoblastic leukemia. Blood 91: 1016-1020.
9. Haviernik, P., et al. 2003. Consistent inactivation of p19 ARF but not p15 INK4B in murine myeloid cells transformed *in vivo* by deregulated c-Myc. Oncogene 22: 1600-1610.

CHROMOSOMAL LOCATION

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

PRODUCT

p16 INK4A/p19 ARF 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 p16 INK4A/p19 ARF shRNA Plasmid (m): sc-43988-SH and p16 INK4A/p19 ARF shRNA (m) Lentiviral Particles: sc-43988-V as alternate gene silencing products.

For independent verification of p16 INK4A/p19 ARF (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-43988A, sc-43988B and sc-43988C.

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

p16 INK4A/p19 ARF siRNA (m) is recommended for the inhibition of p16 INK4A/p19 ARF 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 ARF (5-C3-1): sc-32748 is recommended as a control antibody for monitoring of p16 INK4A/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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor p16 INK4A/p19 ARF gene expression knockdown using RT-PCR Primer: p16 INK4A/p19 ARF (m)-PR: sc-43988-PR (20 µl, 446 bp). 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.