SANTA CRUZ BIOTECHNOLOGY, INC.

p14 ARF/p16 INK4A siRNA (h): sc-37622

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 promotes orderly progression through the cell cycle. Multiple proteins are encoded by the tumor suppressor gene CDKN2A (MTS1/p16 INK4A) via translation of alternate reading frames, resulting in the production of the p19 ARF protein in mice and the p14 ARF/p16 INK4A protein in humans. p14 ARF/p16 INK4A induces an increase in MDM2 and p21 Waf1/Cip1 levels and leads to cell cycle arrest in both G_1 and G_2/M. p14 ARF/p16 INK4A is negatively regulated by p53 and is known to bind directly to MDM2. CDKN2A also encodes the mitotic protein p16 INK4A, which binds to and inhibits the Cdk4/cyclin D complex.

REFERENCES

1. Sherr, C.J. 1993. Mammalian G_1 cyclins. Cell 73: 1059-1065.
2. Hunter, T. 1993. Braking the cycle. Cell 75: 839-841.
3. Larsen, C.J. 1997. Contribution of the dual coding capacity of the p16 INK4A/Mts1/CDKN2 locus to human malignancies. Prog. Cell Cycle Res. 3: 109-124.

CHROMOSOMAL LOCATION

Genetic locus: CDKN2A (human) mapping to 9p21.3.

PRODUCT

p14 ARF/p16 INK4A siRNA (h) 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 p14 ARF/p16 INK4A shRNA Plasmid (h): sc-37622-SH and p14 ARF/p16 INK4A shRNA (h) Lentiviral Particles: sc-37622-V as alternate gene silencing products.

For independent verification of p14 ARF/p16 INK4A (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37622A, sc-37622B and sc-37622C.

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

p14 ARF/p16 INK4A siRNA (h) is recommended for the inhibition of p14 ARF/p16 INK4A expression in human 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

p14 ARF (ARF 4C6/4): sc-53392 is recommended as a control antibody for monitoring of p14 ARF/p16 INK4A 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 p14 ARF/p16 INK4A gene expression knockdown using RT-PCR Primer: p14 ARF/p16 INK4A (h)-PR: sc-37622-PR (20 µl, 412 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Li, L.X., et al. 2013. Antitumor efficacy of a recombinant adenovirus encoding endostatin combined with an E1B55KD-deficient adenovirus in gastric cancer cells. J. Transl. Med. 11: 257.
2. Amir, S., et al. 2013. Oncomir miR-125b suppresses p14 ARF to modulate p53-dependent and p53-independent apoptosis in prostate cancer. PLoS ONE 8: e61064.
3. Jeong, E.H., et al. 2018. Anti-tumor effect of CDK inhibitors on CDKN2A-defective squamous cell lung cancer cells. Cell. Oncol. 41: 663-675.
4. Niu, L.L., et al. 2018. ID1-induced p16/IL6 axis activation contributes to the resistant of hepatocellular carcinoma cells to sorafenib. Cell Death Dis. 9: 852.
5. Rahman, T.U., et al. 2018. Androgen-induced alterations in endometrial proteins crucial in recurrent miscarriages. Oncotarget 9: 24627-24641.
6. Lee, Y.Y., et al. 2020. Mitochondrial nucleoid remodeling and biogenesis are regulated by the p53-^{p21WAF1}-PKCζ pathway in p16 INK4A-silenced cells. Aging 12: 6700-6732.

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.