

# p16 INK4A siRNA (m): sc-36144

## BACKGROUND

The progression of cells through the cell cycle is regulated by a family of protein kinases known as cyclin-dependent kinases (Cdks). The sequential activation of individual members of this family and their consequent phosphorylation of critical substrates promotes orderly progression through the cell cycle. The cyclins function as differentially expressed positive regulators of Cdks. Negative regulators of the cycle include the p53-inducible protein p21 Waf1/Cip1 (also designated p21, WAF1 or Cip1), Kip1 p27 and p16 INK4A. The complexes formed by Cdk4 and the D-type cyclins have been strongly implicated in the control of cell proliferation during the G<sub>1</sub> phase. It has been shown that p16 INK4A binds to Cdk4 and inhibits the catalytic activity of the Cdk4/cyclin D complex. Moreover, the gene encoding p16 INK4A exhibits a high frequency of homozygous deletions and point mutations in established human tumor cell lines.

## REFERENCES

1. Sherr, C.J. 1993. Mammalian G<sub>1</sub> cyclins. *Cell* 73: 1059-1065.
2. Harper, J.W., et al. 1993. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G<sub>1</sub> cyclin-dependent kinases. *Cell* 75: 805-816.
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.

## CHROMOSOMAL LOCATION

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

## PRODUCT

p16 INK4A 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see p16 INK4A shRNA Plasmid (m): sc-36144-SH and p16 INK4A shRNA (m) Lentiviral Particles: sc-36144-V as alternate gene silencing products.

For independent verification of p16 INK4A (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-36144A, sc-36144B and sc-36144C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

p16 INK4A siRNA (m) is recommended for the inhibition of p16 INK4A 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  $\mu$ M in 66  $\mu$ l. 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

p16 INK4A (F-12): sc-1661 is recommended as a control antibody for monitoring of 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).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 p16 INK4A gene expression knockdown using RT-PCR Primer: p16 INK4A (m)-PR: sc-36144-PR (20  $\mu$ l, 419 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Kim, J. and Wong, P.K. 2009. Oxidative stress is linked to ERK1/2-p16 signaling-mediated growth defect in ATM-deficient astrocytes. *J. Biol. Chem.* 284: 14396-16404.
2. Tong, X. and Pelling, J.C. 2009. Enhancement of p53 expression in keratinocytes by the bioflavonoid apigenin is associated with RNA-binding protein HuR. *Mol. Carcinog.* 48: 118-129.
3. Zagon, I.S., et al. 2011. T lymphocyte proliferation is suppressed by the opioid growth factor ([Met(5)]-enkephalin)-opioid growth factor receptor axis: implication for the treatment of autoimmune diseases. *Immunobiology* 216: 579-590.
4. Alessio, N., et al. 2017. Misidentified human gene functions with mouse models: the case of the retinoblastoma gene family in senescence. *Neoplasia* 19: 781-790.

## RESEARCH USE

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