

p27 Kip1 siRNA (h2): sc-44215

BACKGROUND

Cell cycle progression is regulated by a series of cyclin-dependent kinases consisting of catalytic subunits, designated Cdk, as well as activating subunits, designated cyclins. Orderly progression through the cell cycle requires the activation and inactivation of different cyclin-Cdk at appropriate times. A series of proteins has recently been described that function as "mitotic inhibitors". These include p21, the levels of which are elevated upon DNA damage in G₁ in a p53-dependent manner; p16; and a more recently described p16-related inhibitor designated p15. A p21-related protein, p27 Kip1, has been described as a negative regulator of G₁ progression and speculated to function as a possible mediator of TGFβ-induced G₁ arrest. p27 Kip1 interacts strongly with D-type cyclins and Cdk4 *in vitro* and, to a lesser extent, with cyclin E and Cdk2.

REFERENCES

1. Sherr, C.J. 1993. Mammalian G₁ cyclins. *Cell* 73: 1059-1065.
2. El-Deiry, W.S., et al. 1993. WAF1, a potential mediator of p53 tumor suppression. *Cell* 75: 817-825.
3. Xiong, Y., et al. 1993. p21 is a universal inhibitor of cyclin kinases. *Nature* 366: 701-704.
4. Serrano, M., et al. 1993. A new regulatory motif in cell cycle control causing specific inhibition of cyclin D/Cdk4. *Nature* 366: 704-707.
5. Hannon, G.J., et al. 1994. p15^{INK4B} is a potential effector of TGFβ-induced cell cycle arrest. *Nature* 371: 257-260.
6. Hengst, L., et al. 1994. A cell cycle-regulated inhibitor of cyclin-dependent kinases. *Proc. Natl. Acad. Sci. USA* 91: 5291-5295.

CHROMOSOMAL LOCATION

Genetic locus: CDKN1B (human) mapping to 12p13.1.

PRODUCT

p27 Kip1 siRNA (h2) 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 p27 Kip1 shRNA Plasmid (h2): sc-44215-SH and p27 Kip1 shRNA (h2) Lentiviral Particles: sc-44215-V as alternate gene silencing products.

For independent verification of p27 Kip1 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44215A, sc-44215B and sc-44215C.

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

p27 Kip1 siRNA (h2) is recommended for the inhibition of p27 Kip1 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

p27 Kip1 (F-8): sc-1641 is recommended as a control antibody for monitoring of p27 Kip1 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 p27 Kip1 gene expression knockdown using RT-PCR Primer: p27 Kip1 (h2)-PR: sc-44215-PR (20 μl, 482 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wang, Z., et al. 2010. Persistent p21Cip1 induction mediates G₁ cell cycle arrest by methylseleninic acid in DU145 prostate cancer cells. *Curr. Cancer Drug Targets* 10: 307-318.

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.