

p21 Waf1/Cip1 siRNA (r): sc-108036

BACKGROUND

It is now well established that cyclins play a positive role in promoting cell cycle transitions via their ability to associate with and activate their cognate cyclin-dependent kinases (Cdks). Cdk2 associates with cyclins A, D and E, and has been implicated in the control of the G₁ to S phase transition in mammals. A novel Cdk-interacting protein, designated p21 Waf1/Cip1, Cip1 or WAF1, has been identified in cyclin A, cyclin D1, cyclin E and Cdk2 immunoprecipitates. p21 Waf1/Cip1 is a potent, tight-binding inhibitor of Cdks and can inhibit the phosphorylation of Rb by cyclin A-Cdk 2, cyclin E-Cdk2, cyclin D1-Cdk4 and cyclin D2-Cdk4 complexes. Expression of p21 Waf1/Cip1 is inducible by wildtype, but not mutant, p53. The mouse homolog of p21 Waf1/Cip1 is designated CAP20.

REFERENCES

1. Sherr, C.J. 1993. Mammalian G₁ cyclins. *Cell* 73: 1059-1065.
2. Harper, J.W., et al. 1993. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G₁ 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.
5. Kato, J., et al. 1993. Direct binding of cyclin D to the retinoblastoma gene product and pRb phosphorylation by the cyclin D-dependent kinase Cdk4. *Genes Dev.* 7: 331-342.
6. Xiong, Y., et al. 1993. p21 is a universal inhibitor of cyclin kinases. *Nature* 366: 701-704.

CHROMOSOMAL LOCATION

Genetic locus: Cdkn1a (rat) mapping to 20p12.

PRODUCT

p21 Waf1/Cip1 siRNA (r) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see p21 Waf1/Cip1 shRNA Plasmid (r): sc-108036-SH and p21 Waf1/Cip1 shRNA (r) Lentiviral Particles: sc-108036-V as alternate gene silencing products.

For independent verification of p21 Waf1/Cip1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-108036A, sc-108036B and sc-108036C.

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

APPLICATIONS

p21 Waf1/Cip1 siRNA (r) is recommended for the inhibition of p21 Waf1/Cip1 expression in rat 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 μ M in 66 μ 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

p21 Waf1/Cip1 (F-5): sc-6246 is recommended as a control antibody for monitoring of p21 Waf1/Cip1 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 p21 Waf1/Cip1 gene expression knockdown using RT-PCR Primer: p21 Waf1/Cip1 (r)-PR: sc-108036-PR (20 μ l, 588 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Chien, M.H., et al. 2010. β -sitosterol inhibits cell cycle progression of rat aortic smooth muscle cells through increases of p21^{cip1} protein. *J. Agric. Food Chem.* 58: 10064-10069.
2. Sung, J.Y., et al. 2011. AMPK induces vascular smooth muscle cell senescence via LKB1 dependent pathway. *Biochem. Biophys. Res. Commun.* 413: 143-148.
3. Tong, Y.F., et al. 2017. Cyclin-dependent kinase inhibitor p21 Waf1/Cip1 facilitates the development of cardiac hypertrophy. *Cell. Physiol. Biochem.* 42: 1645-1656.

RESEARCH USE

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