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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

p21 siRNA (h2): sc-44214

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, Cip1 or WAF1, has been identified in cyclin A, cyclin D1, cyclin E and Cdk2 immunoprecipitates. p21 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 is inducible by wildtype, but not mutant, p53. The mouse homolog of p21 is designated CAP20.

REFERENCES

- Sherr, C.J. 1993. Mammalian G₁ cyclins. *Cell* 73: 1059-1065.
- 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.
- El-Deiry, W.S., et al. 1993. WAF1, a potential mediator of p53 tumor suppression. *Cell* 75: 817-825.
- Hunter, T. 1993. Braking the cycle. *Cell* 75: 839-841.
- 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.
- Xiong, Y., et al. 1993. p21 is a universal inhibitor of cyclin kinases. *Nature* 366: 701-704.

CHROMOSOMAL LOCATION

Genetic locus: CDKN1A (human) mapping to 6p21.2.

PRODUCT

p21 siRNA (h2) is a pool of 2 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 shRNA Plasmid (h2): sc-44214-SH and p21 shRNA (h2) Lentiviral Particles: sc-44214-V as alternate gene silencing products.

For independent verification of p21 (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-44214A and sc-44214B.

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 siRNA (h2) is recommended for the inhibition of p21 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 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 (F-5): sc-6246 is recommended as a control antibody for monitoring of p21 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 gene expression knockdown using RT-PCR Primer: p21 (h2)-PR: sc-44214-PR (20 μ l, 515 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Doller, A. 2008. Posttranslational modification of the AU-rich element binding protein HuR by protein kinase C δ elicits angiotensin II-induced stabilization and nuclear export of cyclooxygenase 2 mRNA. *Mol. Cell. Biol.* 28: 2608-2625.
- Wang, Z., et al. 2010. Persistent p21^{Cip1} induction mediates G₁ cell cycle arrest by methylseleninic acid in DU145 prostate cancer cells. *Curr. Cancer Drug Targets* 10: 307-318.
- Li, C. and Johnson, D.E. 2013. Liberation of functional p53 by proteasome inhibition in human papilloma virus-positive head and neck squamous cell carcinoma cells promotes apoptosis and cell cycle arrest. *Cell Cycle* 12: 923-934.

RESEARCH USE

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