SANTA CRUZ BIOTECHNOLOGY, INC.

CDKN2AIP siRNA (h): sc-88879

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The Power to Question

BACKGROUND

Cell cycle progression is controlled in part by a family of cyclin proteins and cyclin dependent kinases (Cdks). CDKN2AIP (CDKN2A-interacting protein), also known as CARF, is a 580 amino acid protein that activates p53 via $p14^{ARF}$ (alternate reading frame)-dependent and independent pathways. CDKN2AIP-dependent activation of p53, a protein that upregulates growth arrest and apoptosis-related genes in response to stress signals, leads to an enhancement of p53 function. Expression levels of CDKN2AIP and p53 show an inverse relationship that is caused by a negative-feedback control via a proteasome-mediated degradation pathway. CDKN2AIP is expressed ubiquitously across tissue samples and, along with $p14^{ARF}$, is localized to the perinucleolar region within the nucleus. Through direct interaction with MDM2, CDKN2AIP functions as a repressor of MDM2 transcription and undergoes degradation by the MDM2-dependent proteasome pathway. CDKN2AIP contains one DRBM (double-stranded RNA-binding) domain, suggesting a possible role in posttranscriptional gene regulation.

REFERENCES

1. Hasan, M.K., Yaguchi, T., Sugihara, T., Kumar, P.K., Taira, K., Reddel, R.R., Kaul, S.C. and Wadhwa, R. 2002. CARF is a novel protein that cooperates with mouse $p19^{ARF}$ (human $p14^{ARF}$) in activating p53. J. Biol. Chem. 277: 37765-37770.
2. Hasan, M.K., Yaguchi, T., Minoda, Y., Hirano, T., Taira, K., Wadhwa, R. and Kaul, S.C. 2004. Alternative reading frame protein (ARF)-independent function of CARF (collaborator of ARF) involves its interactions with p53: evidence for a novel p53-activation pathway and its negative feedback control. Biochem. J. 380: 605-610.
3. Kaul, S.C., Hasan, K. and Wadhwa, R. 2006. CARF regulates $p19^{ARF}$-p53-$p21^{WAF1}$ senescence pathway by multiple checkpoints. Ann. N.Y. Acad. Sci. 1067: 217-219.
4. Kamrul, H.M., Wadhwa, R. and Kaul, S.C. 2007. CARF binds to three members (ARF, p53 and HDM2) of the p53 tumor-suppressor pathway. Ann. N.Y. Acad. Sci. 1100: 312-315.

CHROMOSOMAL LOCATION

Genetic locus: CDKN2AIP (human) mapping to 4q35.1.

PRODUCT

CDKN2AIP 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 CDKN2AIP shRNA Plasmid (h): sc-88879-SH and CDKN2AIP shRNA (h) Lentiviral Particles: sc-88879-V as alternate gene silencing products.

For independent verification of CDKN2AIP (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-88879A, sc-88879B and sc-88879C.

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

CDKN2AIP siRNA (h) is recommended for the inhibition of CDKN2AIP 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

CDKN2AIP (18.7): sc-81841 is recommended as a control antibody for monitoring of CDKN2AIP 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 CDKN2AIP gene expression knockdown using RT-PCR Primer: CDKN2AIP (h)-PR: sc-88879-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.