SANTA CRUZ BIOTECHNOLOGY, INC.

Pirh2 siRNA (h): sc-45424

BACKGROUND

Pirh2, also known as androgen receptor N-terminal-interacting protein (ARNIP), ZN363 or CHIMP, has p53-induced ubiquitin-protein ligase activity, promoting p53 degradation. The protein physically interacts with p53 and the resulting degradation of p53 renders Pirh2 an oncogenic protein, as the loss of p53 function contributes to malignant tumor development. The gene encoding for the protein maps to chromosome 4q21.1; transcription of this gene is regulated by p53. Pirh2 expression decreases the level of p53 and a decrease of endogenous Pirh2 expression increases p53 levels. Pirh2 is therefore considered, together with MDM2, to act as a negative regulator of p53 function.

REFERENCES

1. Beitel, L.K., et al. 2002. Cloning and characterization of an androgen receptor N-terminal-interacting protein with ubiquitin-protein ligase activity. J. Mol. Endocrinol. 29: 41-60.
2. Leng, R.P., et al. 2003. Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. Cell 112: 779-791.
3. Duan, W., et al. 2004. Expression of Pirh2, a newly identified ubiquitin protein ligase, in lung cancer. J. Natl. Cancer Inst. 96: 1718-1721.
4. Corcoran, C.A., et al. 2004. The p53 paddy wagon: COP1, Pirh2 and MDM2 are found resisting apoptosis and growth arrest. Cancer Biol. Ther. 3: 721-725.
5. Dornan, D., et al. 2004. The ubiquitin ligase COP1 is a critical negative regulator of p53. Nature 429: 86-92.

CHROMOSOMAL LOCATION

Genetic locus: RCHY1 (human) mapping to 4q21.1.

PRODUCT

Pirh2 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 Pirh2 shRNA Plasmid (h): sc-45424-SH and Pirh2 shRNA (h) Lentiviral Particles: sc-45424-V as alternate gene silencing products.

For independent verification of Pirh2 (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-45424A, sc-45424B and sc-45424C.

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

Pirh2 siRNA (h) is recommended for the inhibition of Pirh2 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

Pirh2 (D-12): sc-374505 is recommended as a control antibody for monitoring of Pirh2 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 Pirh2 gene expression knockdown using RT-PCR Primer: Pirh2 (h)-PR: sc-45424-PR (20 µl, 519 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Duan, S., et al. 2007. Phosphorylation of Pirh2 by calmodulin-dependent kinase II impairs its ability to ubiquitinate p53. EMBO J. 26: 3062-3074.
2. Min, S.H., et al. 2009. New p53 target, phosphatase of regenerating liver 1 (PRL-1) downregulates p53. Oncogene 28: 545-554.
3. Yang-Hartwich, Y., et al. 2019. p53-Pirh2 complex promotes Twist1 degradation and inhibits EMT. Mol. Cancer Res. 17: 153-164.

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.