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PARC siRNA (h): sc-44715

BACKGROUND

The p53 tumor suppressor gene is altered in over 50% of human cancers. The p53 binding proteins 53BP1 and 53BP2 (Bbp) are tumor suppressors that bind to the site-specific central DNA-binding domain of wild type p53 in a conformation-dependent manner. Severe DNA damage can cause phosphorylation of p53 at position Serine 46. This event triggers expression of p53AIP1 (apoptosis inducing protein), which contributes to subsequent events leading to programmed cell death. The protein PARC (p53 associated Parkin-like cytoplasmic protein) acts as a cytoplasmic anchor for p53 in unstressed cells, thereby regulating the localization and subsequent function of p53. The C-terminus of the PARC protein contains a RING-IBR-RING domain, which suggests it retains ubiquitin ligase activity, but PARC fails to promote degradation of p53. The gene encoding human PARC maps to chromosome 6p21.1.

REFERENCES

1. Iwabuchi, K., Bartel, P., Li, B., Marraccino, R. and Fields, S. 1994. Two cellular proteins that bind to wild type but not mutant p53. *Proc. Natl. Acad. Sci. USA* 91: 6098-6102.
2. Iwabuchi, K., Li, B., Massa, H.F., Trask, B.J., Date, T. and Fields, S. 1998. Stimulation of p53-mediated transcriptional activation by the p53-binding proteins, 53BP1 and 53BP2. *J. Biol. Chem.* 273: 26061-26068.
3. Oda, K., Arakawa, H., Tanaka, T., Matsuda, K., Tanikawa, C., Mori, T., Nishimori, H., Tamai, K., Tokino, T., Nakamura, Y. and Taya, Y. 2000. p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser 46-phosphorylated p53. *Cell* 102: 849-862.
4. Nikolaev, A.Y., Li, M., Puskas, N., Qin, J. and Gu, W. 2003. PARC: a cytoplasmic anchor for p53. *Cell* 112: 29-40.

CHROMOSOMAL LOCATION

Genetic locus: CUL9 (human) mapping to 6p21.1.

PRODUCT

PARC 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PARC shRNA Plasmid (h): sc-44715-SH and PARC shRNA (h) Lentiviral Particles: sc-44715-V as alternate gene silencing products.

For independent verification of PARC (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-44715A, sc-44715B and sc-44715C.

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

PARC siRNA (h) is recommended for the inhibition of PARC expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29527 (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

PARC (NQ-C32): sc-134412 is recommended as a control antibody for monitoring of PARC 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 PARC gene expression knockdown using RT-PCR Primer: PARC (h)-PR: sc-44715-PR (20 μ l, 551 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Woo, M.G., Xue, K., Liu, J., McBride, H. and Tsang, B.K. 2012. Calpain-mediated processing of p53-associated parkin-like cytoplasmic protein (PARC) affects chemosensitivity of human ovarian cancer cells by promoting p53 subcellular trafficking. *J. Biol. Chem.* 287: 3963-3975.

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.