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PARC shRNA (h) Lentiviral Particles: sc-44715-V

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

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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.
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CHROMOSOMAL LOCATION

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

PRODUCT

PARC shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see PARC siRNA (h): sc-44715 and PARC shRNA Plasmid (h): sc-44715-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

PARC shRNA (h) Lentiviral Particles is recommended for the inhibition of PARC expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.