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BRIP1 shRNA (h) Lentiviral Particles: sc-43640-V

BACKGROUND

Genes that contribute to tumorigenesis can be broadly classified as either gatekeepers or caretakers. Genes in the gatekeeper class directly regulate cell division or cell death, and their alteration results in the uncontrolled cellular proliferation that characterizes tumor cells. Genes in the caretaker class are involved in DNA metabolic processes and are responsible for maintaining the overall stability of the genome. An unusual mutator phenotype in *Caenorhabditis elegans*, characterized by deletions that start around the 3' end of polyguanine tracts and terminate at variable positions 5' from such tracts, results from disruption of a gene that encodes BRIP1 (also designated BACH1 or BRCA1-associated carboxy-terminal helicase-1). BRCA1 interacts *in vivo* with BRIP1, a member of the DEAH helicase family. BRIP1 contains the seven helicase-specific motifs that are conserved among members of the DEAH family, and the helicase domain includes a nuclear localization signal. BRIP1 is ubiquitously expressed with highest levels in testis, an expression pattern similar to that of BRCA1. BRIP1 binds directly to the BRCT repeats of BRCA1 and the BRIP1-BRCA1 complex formation contributes to a key BRCA1 activity. BRIP1 is required to resolve the secondary structures of guanine-rich DNA that occasionally form during lagging-strand DNA synthesis. Phosphorylated BRIP1/BACH1 binds directly to the BRCT domain of BRCA1. This interaction is dependent on the phosphorylation of BRIP1/BACH1 at Ser 990, and is required for DNA damage-induced checkpoint control during the G₂ to M phase transition of the cell cycle.

REFERENCES

1. Cantor, S.B., et al. 2001. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell* 105: 149-160.
2. Liu, Y., et al. 2002. Distinct functions of BRCA1 and BRCA2 in double-strand break repair. *Breast Cancer Res.* 4: 9-13.

CHROMOSOMAL LOCATION

Genetic locus: BRIP1 (human) mapping to 17q23.2.

PRODUCT

BRIP1 shRNA (h) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁹ 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 BRIP1 siRNA (h): sc-43640 and BRIP1 shRNA Plasmid (h): sc-43640-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

BRIP1 shRNA (h) Lentiviral Particles is recommended for the inhibition of BRIP1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

BRIP1 (E-11): sc-365708 is recommended as a control antibody for monitoring of BRIP1 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 BRIP1 gene expression knockdown using RT-PCR Primer: BRIP1 (h)-PR: sc-43640-PR (20 µl, 451 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.