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BRD4 shRNA (h) Lentiviral Particles: sc-43639-V

BACKGROUND

Brd4 belongs to the BET family, a group of structurally related proteins containing two bromodomains. Through these two domains, Brd4 associates with mitotic chromosomes and its expression correlates with cell growth. Expression of Brd4 inhibits cell cycle progression from G₁ to S, due to binding to the largest subunit of replication factor C (RFC) to prevent DNA elongation. Altered Brd4 function correlates with poorly differentiated carcinoma, with aggressive phenotype and a highly lethal outcome.

REFERENCES

1. French, C.A., et al. 2001. BRD4 bromodomain gene rearrangement in aggressive carcinoma with translocation t(15;19). *Am. J. Pathol.* 159: 1987-1992.
2. Houzelstein, D., et al. 2002. Growth and early postimplantation defects in mice deficient for the bromodomain-containing protein BRD4. *Mol. Cell. Biol.* 22: 3794-3802.
3. Maruyama, T., et al. 2002. A mammalian bromodomain protein, BRD4, interacts with replication factor C and inhibits progression to S phase. *Mol. Cell. Biol.* 22: 6509-6520.
4. French, C.A., et al. 2003. BRD4-NUT fusion oncogene: a novel mechanism in aggressive carcinoma. *Cancer Res.* 63: 304-307.
5. You, J., et al. 2004. Interaction of the bovine papillomavirus E2 protein with BRD4 tethers the viral DNA to host mitotic chromosomes. *Cell* 117: 349-360.
6. LocusLink Report (LocusID: 23476). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: BRD4 (human) mapping to 19p13.12.

PRODUCT

BRD4 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 x 10⁶ 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 BRD4 siRNA (h): sc-43639 and BRD4 shRNA Plasmid (h): sc-43639-SH as alternate gene silencing products.

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.

APPLICATIONS

BRD4 shRNA (h) Lentiviral Particles is recommended for the inhibition of BRD4 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l 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

BRD4 (H-250): sc-48772 is recommended as a control antibody for monitoring of BRD4 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor BRD4 gene expression knockdown using RT-PCR Primer: BRD4 (h)-PR: sc-43639-PR (20 μ l, 598 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.

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.