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# B7-H3 shRNA (h) Lentiviral Particles: sc-45444-V

## BACKGROUND

T cell activation and immune function are regulated by the innate immune system through positive and negative costimulatory molecules. One such molecule, B7-H3 (B7-homolog 3, also designated B7RP-2) belongs to the B7 immunoglobulin superfamily. Soluble B7-H3 binds a putative receptor on activated T cells that is distinct from CD28, CTLA-4, ICOS and PD-1. Widely expressed on nonlymphoid tissues, B7-H3 costimulates proliferation of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells. The ability of B7-H3 to stimulate Th1 and cytotoxic-T cell responses suggest that it may have antitumor activity. B7-H3 interactions may play a role in regulating cell-mediated immune responses against cancer, implicating B7-H3 as a potential therapeutic tool.

## REFERENCES

1. Chapoval, A.I., et al. 2001. B7-H3: a costimulatory molecule for T cell activation and IFN- $\gamma$  production. *Nat. Immunol.* 2: 269-274.
2. Ferlazzo, G., et al. 2002. T lymphocytes express B7 family molecules following interaction with dendritic cells and acquire bystander costimulatory properties. *Eur. J. Immunol.* 32: 3092-3101.
3. Sun, M., et al. 2002. Characterization of mouse and human B7-H3 genes. *J. Immunol.* 168: 6294-6297.
4. Suh, W.K., et al. 2003. The B7 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses. *Nat. Immunol.* 4: 899-906.
5. Sun, X., et al. 2003. Mouse B7-H3 induces antitumor immunity. *Gene Ther.* 10: 1728-1734.
6. Prasad, D.V., et al. 2004. Murine B7-H3 is a negative regulator of T cells. *J. Immunol.* 173: 2500-2506.
7. Suh, W.K., et al. 2004. The immune regulatory protein B7-H3 promotes osteoblast differentiation and bone mineralization. *Proc. Natl. Acad. Sci. USA* 101: 12969-12973.

## CHROMOSOMAL LOCATION

Genetic locus: CD276 (human) mapping to 15q24.1.

## PRODUCT

B7-H3 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see B7-H3 siRNA (h): sc-45444 and B7-H3 shRNA Plasmid (h): sc-45444-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.

## APPLICATIONS

B7-H3 shRNA (h) Lentiviral Particles is recommended for the inhibition of B7-H3 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

B7-H3 (D-15): sc-34569 is recommended as a control antibody for monitoring of B7-H3 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor B7-H3 gene expression knockdown using RT-PCR Primer: B7-H3 (h)-PR: sc-45444-PR (20  $\mu$ l, 553 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.

## PROTOCOLS

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