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Fap-1 shRNA (h) Lentiviral Particles: sc-43560-V

BACKGROUND

In contrast to the growth factors which promote cell proliferation, FAS ligand (FAS-L) and the tumor necrosis factors (TNFs) rapidly induce apoptosis. Cellular response to FAS-L and TNF is mediated by structurally-related receptors containing a conserved "death domain" belonging to the TNF receptor superfamily. Putative downstream receptors of FAS include TRADD, FADD and RIP. A novel protein tyrosine phosphatase, Fap-1 (for FAS-associated phosphatase) (originally designated PTP-BAS), has been shown to associate with the carboxy terminus fifteen amino acids of FAS. Three isoforms of the protein result from alternative RNA splicings, the longest of which encodes a protein 2485 amino acids in length. Although lacking a transmembrane region, Fap-1 does contain a membrane-binding domain, similar to that found in cytoskeleton-associated proteins such as ezrin. Fap-1 does not seem to associate with CD40 or death domain proteins such as TNF-RI and TNF-RII.

REFERENCES

1. Maekawa, K., et al. 1994. Molecular cloning of a novel protein tyrosine phosphatase containing a membrane-binding domain and GLGF repeats. *FEBS Lett.* 337: 200-206.
2. Moller, N.P., et al. 1994. Src kinase associates with a member of a distinct subfamily of protein-tyrosine phosphatases containing an ezrin-like domain. *Proc. Natl. Acad. Sci. USA* 91: 7477-7481.
3. Cleveland, J.L., et al. 1995. Contenders in FasL/TNF death signaling. *Cell* 81: 479-482.
4. Hsu, H., et al. 1995. The TNF receptor 1-associated protein TRADD signals cell death and NF κ B activation. *Cell* 81: 495-504.
5. Chinnaiyan, A.M., et al. 1995. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 81: 505-512.
6. Stanger, B.Z., et al. 1995. RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. *Cell* 81: 513-523.

CHROMOSOMAL LOCATION

Genetic locus: PTPN13 (human) mapping to 4q21.3.

PRODUCT

Fap-1 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 Fap-1 siRNA (h): sc-43560 and Fap-1 shRNA Plasmid (h): sc-43560-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

Fap-1 shRNA (h) Lentiviral Particles is recommended for the inhibition of Fap-1 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

Fap-1 (H-300): sc-15356 is recommended as a control antibody for monitoring of Fap-1 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 Fap-1 gene expression knockdown using RT-PCR Primer: Fap-1 (h)-PR: sc-43560-PR (20 μ l, 398 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.

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