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SANTA CRUZ BIOTECHNOLOGY, INC.

FLASH shRNA (h) Lentiviral Particles: sc-43761-V

BACKGROUND

Fas is a member of the tumor necrosis factor family of membrane receptors, which induces apoptosis by binding to its ligand, Fas-L. Fas mediates apoptosis through a group of proteins that bind to its intracellular "death" domain, including FADD. After binding to Fas, FADD binds to caspase-8, resulting in activation of caspase-8 and the initiation of the caspase-mediated apoptotic pathway. FLASH, for FLICE- associated huge protein, has been identified as an additional component of the Fas-FADD-caspase-8 complex, also referred to as the DISC complex. FLASH shares homology with the *C. elegans* CED-4 protein and the mammalian Apaf-1 protein, which are both involved in activating caspases. FLASH was shown to be required for activation of caspase-8 during Fas-mediated apoptosis.

REFERENCES

1. Itoh, N., et al. 1991. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 66: 233-243.
2. Suda, T., et al. 1993. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell 75: 1169-1178.
3. 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.
4. Boldin, M.P., et al. 1995. A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. J. Biol. Chem. 270: 7795-7798.
5. Boldin, M.P., et al. 1996. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell 85: 803-815.

CHROMOSOMAL LOCATION

Genetic locus: CASP8AP2 (human) mapping to 6q15.

PRODUCT

FLASH 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 µl frozen stock containing 1.0 x 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 FLASH siRNA (h): sc-43761 and FLASH shRNA Plasmid (h): sc-43761-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

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

SUPPORT REAGENTS

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

FLASH (M-300): sc-9088 is recommended as a control antibody for monitoring of FLASH 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 FLASH gene expression knockdown using RT-PCR Primer: FLASH (h)-PR: sc-43761-PR (20 µl, 455 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.