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FAS shRNA (h2) Lentiviral Particles: sc-44260-V

BACKGROUND

Cytotoxic T lymphocyte (CTL)-mediated cytotoxicity constitutes an important component of specific effector mechanisms in immunosurveillance against virus-infected or transformed cells. Two mechanisms appear to account for this activity, one of which is the perforin-based process. Independently, a FAS-based mechanism involves the transducing molecule FAS (also designated APO-1) and its ligand (FAS-L). The human FAS (APO-1) protein is a cell surface glycoprotein that belongs to a family of receptors that includes CD40, nerve growth factor receptors and tumor necrosis factor receptors. The FAS antigen is expressed on a broad range of lymphoid cell lines, certain of which undergo apoptosis in response to treatment with antibody to FAS. These findings strongly imply that targeted cell death is potentially mediated by the intercellular interactions of FAS with its ligand or effectors, and that FAS may be critically involved in CTL-mediated cytotoxicity.

REFERENCES

- Henkart, P.A. 1985. Mechanism of lymphocyte-mediated cytotoxicity. *Annu. Rev. Immunol.* 3: 31-58.
- Young, J.D.E., et al. 1988. Perforin-dependent and independent pathways of cytotoxicity mediated by lymphocytes. *Immunol. Rev.* 103: 161-202.
- Podack, E.R., et al. 1991. A central role of perforin in cytolysis? *Annu. Rev. Immunol.* 9: 129-157.
- Yagita, H., et al. 1992. Role of perforin in lymphocyte-mediated cytolysis. *Adv. Immunol.* 51: 215-242.
- Drappa, J., et al. 1993. The FAS protein is expressed at high levels on CD4⁺CD8⁺ thymocytes and activated mature lymphocytes in normal mice but not in the lupus-prone strain, MRL lpr/lpr. *Proc. Natl. Acad. Sci. USA* 90: 10340-10344.

CHROMOSOMAL LOCATION

Genetic locus: FAS (human) mapping to 10q23.31.

PRODUCT

FAS shRNA (h2) 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 1.0×10^6 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 FAS siRNA (h2): sc-44260 and FAS shRNA Plasmid (h2): sc-44260-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

FAS shRNA (h2) Lentiviral Particles is recommended for the inhibition of FAS expression in human cells.

SUPPORT REAGENTS

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

FAS (B-10): sc-8009 is recommended as a control antibody for monitoring of FAS 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 FAS gene expression knockdown using RT-PCR Primer: FAS (h)-PR: sc-29311-PR (20 µl). 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.