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FAS siRNA (h2): sc-44260

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

1. Henkart, P.A. 1985. Mechanism of lymphocyte-mediated cytotoxicity. *Annu. Rev. Immunol.* 3: 31-58.
2. Young, J.D.E., et al. 1988. Perforin-dependent and independent pathways of cytotoxicity mediated by lymphocytes. *Immunol. Rev.* 103: 161-202.
3. Podack, E.R., et al. 1991. A central role of perforin in cytolysis? *Annu. Rev. Immunol.* 9: 129-157.
4. Yagita, H., et al. 1992. Role of perforin in lymphocyte-mediated cytolysis. *Adv. Immunol.* 51: 215-242.
5. 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 siRNA (h2) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see FAS shRNA Plasmid (h2): sc-44260-SH and FAS shRNA (h2) Lentiviral Particles: sc-44260-V as alternate gene silencing products.

For independent verification of FAS (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44260A, sc-44260B and sc-44260C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

FAS siRNA (h2) is recommended for the inhibition of FAS expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor FAS gene expression knockdown using RT-PCR Primer: FAS (h2)-PR: sc-29311-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.