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caspase-8 siRNA (r): sc-156166

BACKGROUND

Initiator caspases, which include caspase-8, activate effector caspases by cleaving inactive forms of effector caspases. In the activation cascade responsible for apoptosis induced by TNFRSF1A and mediated by TNFRSF6/Fas, caspase-8 is the most upstream protease. Caspase-8 binds to adaptor molecule FADD, forming an aggregate referred to as death-inducing signaling complex (DISC), which activates caspase-8. The activated protein is released from the complex and further activates downstream apoptotic proteases. Caspase-8, which is a heterodimer consisting of two subunits (p18 and p10), is widely expressed, but is detected at highest levels in peripheral blood leukocytes (PBLs), thymus, liver and spleen. Defects in CASP8, the gene encoding for caspase-8, may cause CASP8D (caspase-8 deficiency disorder), which is characterized by splenomegaly and CD95-induced apoptosis of PBLs, and may lead to immunodeficiency due to defects in T lymphocyte, NK cell and B lymphocyte activation.

REFERENCES

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2. Cleveland, J.L., et al. 1995. Contenders in FasL/TNF death signaling. *Cell* 81: 479-482.
3. Medema, J.P., et al. 1997. FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). *EMBO J.* 16: 2794-2804.
4. Fernandes-Alnemri, T., et al. 1996. *In vitro* activation of CPP32 and Mch3 by Mch4, a novel human apoptotic Cysteine protease containing two FADD-like domains. *Proc. Natl. Acad. Sci. USA* 93: 7464-7469.
5. Srinivasan, A., et al. 1998. Bcl-x_L functions downstream of caspase-8 to inhibit Fas- and TNF-R1-induced apoptosis of MCF7 breast carcinoma cells. *J. Biol. Chem.* 273: 4523-4529.
6. Rytomaa, M., et al. 1999. Involvement of FADD and caspase-8 signalling in detachment-induced apoptosis. *Curr. Biol.* 9: 1043-1046.
7. Wesselborg, S., et al. 1999. Anticancer drugs induce caspase-8/FLICE activation and apoptosis in the absence of CD95 receptor/ligand interaction. *Blood* 93: 3053-3063.
8. Watt, W., et al. 1999. The atomic-resolution structure of human caspase-8, a key activator of apoptosis. *Structure* 7: 1135-1143.

CHROMOSOMAL LOCATION

Genetic locus: Casp8 (rat) mapping to 9q31.

PRODUCT

caspase-8 siRNA (r) 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 caspase-8 shRNA Plasmid (r): sc-156166-SH and caspase-8 shRNA (r) Lentiviral Particles: sc-156166-V as alternate gene silencing products.

For independent verification of caspase-8 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156166A, sc-156166B and sc-156166C.

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

caspase-8 siRNA (r) is recommended for the inhibition of caspase-8 expression in rat 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

caspase-8 p18 (D-8): sc-5263 is recommended as a control antibody for monitoring of caspase-8 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 caspase-8 gene expression knockdown using RT-PCR Primer: caspase-8 (r)-PR: sc-156166-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.