SANTA CRUZ BIOTECHNOLOGY, INC.

DAP-1 siRNA (m): sc-37380

BACKGROUND

In contrast to 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 cytoplasmic region called the "death domain". DAP-1 (for death associated protein-1) is a basic, proline-rich protein expressed in γ interferon (IFN-γ)-induced HeLa cells. DAP-1 is a member of the ubiquitin homology (UbH) family which also includes SUMO-1 and SIII p18 elogin protein. DAP-1 interacts with the death domain of TNF-R1 and can trigger programmed cell death in a variety of cell lines, as well as suppress NFκB/Rel activity.

REFERENCES

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2. Itoh, N., et al. 1993. A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen. J. Biol. Chem. 268: 10932-10937.
3. Smith, C.A., et al. 1994. The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. Cell 76: 959-962.
4. Nagata, S., et al. 1995. The Fas death factor. Science 267: 1449-1456.
5. Deiss, L.P., et al. 1995. Identification of a novel serine/threonine kinase and a novel 15 kDa protein as potential mediators of the γ interferon-induced cell death. Genes Dev. 9: 15-30.
6. Garrett, K.P., et al. 1995. Positive regulation of general transcription factor SIII by a tailed ubiquitin homolog. Proc. Natl. Acad. Sci. USA 92: 7172-7176.
7. Boddy, M.N., et al. 1996. PIC 1, a novel ubiquitin-like protein which interacts with the PML component of a multiprotein complex that is disrupted in acute promyelocytic leukemia. Oncogene 13: 971-982.
8. Ware, C.F., et al. 1996. Apoptosis mediated by the TNF-related cytokine and receptor families. J. Cell. Biochem. 60: 47-55.
9. Liou, M.L., et al. 1999. The ubiquitin-homology protein, DAP-1, associates with tumor necrosis factor receptor (p60) death domain and induces apoptosis. Biol. Chem. 274: 10145-10153.

CHROMOSOMAL LOCATION

Genetic locus: Dap (mouse) mapping to 15 B2.

PRODUCT

DAP-1 siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DAP-1 shRNA Plasmid (m): sc-37380-SH and DAP-1 shRNA (m) Lentiviral Particles: sc-37380-V as alternate gene silencing products.

For independent verification of DAP-1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37380A, sc-37380B and sc-37380C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

DAP-1 siRNA (m) is recommended for the inhibition of DAP-1 expression in mouse 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 µM in 66 µl. 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

DAP-1 (C-8): sc-376754 is recommended as a control antibody for monitoring of DAP-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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 DAP-1 gene expression knockdown using RT-PCR Primer: DAP-1 (m)-PR: sc-37380-PR (20 µl). 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.