



DAP-3 siRNA (h): sc-37381

BACKGROUND

The death-associated protein 3 (DAP-3), is a nucleotide-binding protein that contains a potential P-loop motif. It is a positive mediator of programmed cell death; overexpressed intact full-length protein is required in order to induce apoptosis. DAP-3 functions downstream of its receptor signaling complex and its death promoting effects depend on caspase activity. It also interacts with the glucocorticoid receptor (GR), where its main interaction domain is the amino-terminal region, which acts in a dominant-negative fashion to protect cells from apoptosis. DAP-3 protein may also play a role in modulating the cytoplasmic GR/HSP 90 complex. It is conserved at the functional, as well as the structural level, and is ubiquitously expressed in highly proliferative epithelial compartments of various tissues. Unlike a number of other proteins, DAP-3 retains its mitochondrial localization during the induction of apoptosis.

REFERENCES

1. Kissil, J.L., et al. 1995. Isolation of DAP-3, a novel mediator of interferon- γ -induced cell death. *J. Biol. Chem.* 270: 27932-27936.
2. Levy-Strumpf, N., et al. 1998. Death associated proteins (DAPs): from gene identification to the analysis of their apoptotic and tumor suppressive functions. *Oncogene* 17: 3331-3340.
3. Kissil, J.L., et al. 1999. Structure-function analysis of an evolutionary conserved protein, DAP-3, which mediates TNF α - and Fas-induced cell death. *EMBO J.* 18: 353-362.
4. Kimchi, A. 1999. DAP kinase and DAP-3: novel positive mediators of apoptosis. *Ann. Rheum. Dis.* 58: 114-119.
5. Hulkko, S.M., et al. 2000. The pro-apoptotic protein death-associated protein 3 (DAP-3) interacts with the glucocorticoid receptor and affects the receptor function. *Biochem. J.* 349: 885-893.
6. Berger, T., et al. 2000. The apoptosis mediator mDAP-3 is a novel member of a conserved family of mitochondrial proteins. *J. Cell Sci.* 113: 3603-3612.
7. Mariani, L., et al. 2001. Death-associated protein 3 (DAP-3) is overexpressed in invasive glioblastoma cells *in vivo* and in glioma cell lines with induced motility phenotype *in vitro*. *Clin. Cancer Res.* 7: 2480-2489.

CHROMOSOMAL LOCATION

Genetic locus: DAP3 (human) mapping to 1q22.

PRODUCT

DAP-3 siRNA (h) 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 DAP-3 shRNA Plasmid (h): sc-37381-SH and DAP-3 shRNA (h) Lentiviral Particles: sc-37381-V as alternate gene silencing products.

For independent verification of DAP-3 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37381A, sc-37381B and sc-37381C.

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

DAP-3 siRNA (h) is recommended for the inhibition of DAP-3 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

DAP-3 (F-12): sc-373911 is recommended as a control antibody for monitoring of DAP-3 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 DAP-3 gene expression knockdown using RT-PCR Primer: DAP-3 (h)-PR: sc-37381-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.