

DEDD siRNA (h): sc-37383

BACKGROUND

Apoptosis is a physiological process by which multicellular organisms eliminate unwanted cells. DEDD (death effector domain-containing DNA binding protein) induces apoptosis by triggering a series of intracellular protein-protein interactions mediated by the N-terminal DED motif. DEDD, a cytoplasmic protein, translocates to the nucleus during CD95-mediated apoptosis, where it localizes to nucleoli-like structures, activates caspase-6 and specifically inhibits RNA polymerase I-dependent transcription. The cell death activity of DEDD relates to its nuclear localization. The DED in DEDD is sufficient for its DNA binding, caspase-6 activating and Pol I specific transcriptional repressor activity. Point specific mutations indicate that the DED in DEDD represents a novel domain that is structurally similar to other DEDs but functionally different from classical DEDs found in FADD or caspase-8. DEDD is widely expressed in a variety of tissues, with highest levels in the testis. The human DEDD gene maps to chromosome 1q23.3. Alternative splicing results in two transcript variants which encode the same protein.

REFERENCES

1. Leo, C.P., et al. 1998. DEFT, a novel death effector domain-containing molecule predominantly expressed in testicular germ cells. *Endocrinology* 139: 4839-4848.
2. Stegh, A.H., et al. 1998. DEDD, a novel death effector domain-containing protein, targeted to the nucleolus. *EMBO J.* 17: 5974-5986.
3. Schickling, O., et al. 2001 Nuclear localization of DEDD leads to caspase-6 activation through its death effector domain and inhibition of RNA polymerase I dependent transcription. *Cell Death Differ.* 8: 1157-1168.
4. Alcivar, A., et al. 2004. DEDD and DEDD2 associate with caspase-8/10 and signal cell death. *Oncogene* 22: 291-297.
5. LocusLink Report (LocusID: 9191). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: DEDD (human) mapping to 1q23.3.

PRODUCT

DEDD 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 DEDD shRNA Plasmid (h): sc-37383-SH and DEDD shRNA (h) Lentiviral Particles: sc-37383-V as alternate gene silencing products.

For independent verification of DEDD (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-37383A, sc-37383B and sc-37383C.

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

DEDD siRNA (h) is recommended for the inhibition of DEDD 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

DEDD (H-4): sc-271192 is recommended as a control antibody for monitoring of DEDD 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 DEDD gene expression knockdown using RT-PCR Primer: DEDD (h)-PR: sc-37383-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.