SANTA CRUZ BIOTECHNOLOGY, INC.

TID-$1_{L/S}$ siRNA (h): sc-36673

BACKGROUND

TID-1 is the human homolog of the *Drosophila* tumor suppressor protein, TID56. Both TID56 and TID-1 belong to the DnaJ family of proteins which are characterized by a highly conserved J domain that influence apoptotic activity. The human TID-1 gene encodes two splice variants, TID-1_L and TID-1_S. TID-1_L expression increases apoptosis, whereas a mutant J domain suppresses apoptosis. By contrast, TID-1_S expression suppresses apoptosis, whereas a mutant J domain increases apoptosis. TID-1_L and TID-1_S are localized to the mitochondrial matrix where they regulate apoptotic signal transduction by affecting cytochrome c release and caspase-3 activation. Both TID-1_L and TID-1_S are cleaved at amino acid 66 upon entry into the mitochondria, indicating that mature TID-1_L and TID-1_S represent cleavage products of cytoplasmic pre-proteins.

REFERENCES

1. Kurzik-Dumke, U., et al. 1995. Tumor suppression in *Drosophila* is causally related to the funciton of the lethal2 tumorous imaginal discs gene, a DnaJ homolog. Dev. Genet. 16: 64-76.
2. Schilling, B., et al. 1998. A novel human DnaJ protein, hTID-1, a homolog of the *Drosophila* tumor suppressor protein TID56, can interact wiht the human papillomavirus type 16 E7 oncoprotein. Virology 247: 74-85.
3. Bukau, B. and Horwich, A. 1998. The HSP 70 and HSP 60 chaperone machines. Cell 92: 351-366.
4. Green, D. and Reed, D. 1998. Mitochondria and apoptosis. Science 281: 1309-1312.
5. Syken, J., et al. 1999. TID-1, a human homolog of the *Drosophila* tumor suppressor l2tid, encodes two mitochondrial modulators of apoptosis with opposing functions. Proc. Natl. Acad. Sci. USA 96: 8499-8504.

CHROMOSOMAL LOCATION

Genetic locus: DNAJA3 (human) mapping to 16p13.3.

PRODUCT

TID-$1_{L/S}$ 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TID-$1_{L/S}$ shRNA Plasmid (h): sc-36673-SH and TID-$1_{L/S}$ shRNA (h) Lentiviral Particles: sc-36673-V as alternate gene silencing products.

For independent verification of TID-$1_{L/S}$ (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-36673A, sc-36673B and sc-36673C.

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 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

TID-$1_{L/S}$ siRNA (h) is recommended for the inhibition of TID-$1_{L/S}$ 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 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

TID-$1_{L/S}$ (RS-13): sc-18819 is recommended as a control antibody for monitoring of TID-$1_{L/S}$ 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 TID-$1_{L/S}$ gene expression knockdown using RT-PCR Primer: TID-$1_{L/S}$ (h)-PR: sc-36673-PR (20 µl, 564 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.