SANTA CRUZ BIOTECHNOLOGY, INC.

DAP-5 siRNA (h): sc-35169

BACKGROUND

Death-associated protein 5 (DAP-5) (also known as p97 and NAT1) is a member of the eukaryotic translation initiation factor-4G (eIF4G) family. DAP-5 is ubiquitously expressed and is highly conserved among species. In response to activated Fas or p53, caspase cleaves DAP-5 at position 790 to yield a C-terminal truncated protein, which is capable of forming complexes with eIF4A and eIF3. DAP-5 has homology to the carboxy-terminal portion of eIF4G, but lacks the N-terminal region of eIF4G, which is responsible for association with the CAP binding protein eIF4E. By forming translationally inactive complexes with eIF4A and eIF3, but not with eIF4E, DAP-5 functions as a general repressor of translation. During apotosis, the caspase-activated DAP-5 can mediate CAP-independent translation at least from its own internal ribosome entry site, thus resulting in a positive feedback loop responsible for the continuous translation of DAP-5. DAP-5 is also required for cellular differentiation, as it controls specific gene expression pathways.

REFERENCES

1. Levy-Strumpf, N., et al. 1997. DAP-5, a novel homolog of eukaryotic translation initiation factor 4G isolated as a putative modulator of γ interferon-induced programmed cell death. Mol. Cell. Biol. 17: 1615-1625.
2. Yamanaka, S., et al. 1997. A novel translational repressor mRNA is edited extensively in livers containing tumors caused by the transgene expression of the apoB mRNA-editing enzyme. Genes Dev. 11: 321-333.
3. Imataka, H., et al. 1997. A new translational regulator with homology to eukaryotic translation initiation factor 4G. EMBO J. 16: 817-825.
4. 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.
5. Henis-Korenblit, S., et al. 2000. A novel form of DAP5 protein accumulate in apoptotic cells as a result of caspase cleavage and internal ribosome entry site-mediated translation. Mol. Cell. Biol. 20: 496-506.
6. Yamanaka, S., et al. 2000. Essential role of NAT1/p97/DAP-5 in embryonic differentiation and the retinoic acid pathway. EMBO J. 19: 5533-5541.
7. Wittke, I. et al. 2001. DAP-5 is involved in MycN/IFNγ-induced apoptosis in human neuroblastoma cells. Cancer Lett. 162: 237-243.

CHROMOSOMAL LOCATION

Genetic locus: EIF4G2 (human) mapping to 11p15.3.

PRODUCT

DAP-5 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 DAP-5 shRNA Plasmid (h): sc-35169-SH and DAP-5 shRNA (h) Lentiviral Particles: sc-35169-V as alternate gene silencing products.

For independent verification of DAP-5 (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-35169A, sc-35169B and sc-35169C.

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-5 siRNA (h) is recommended for the inhibition of DAP-5 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

DAP-5 (F-2): sc-137011 is recommended as a control antibody for monitoring of DAP-5 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-5 gene expression knockdown using RT-PCR Primer: DAP-5 (h)-PR: sc-35169-PR (20 µl, 443 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Hanson, P.J., et al. 2016. Cleavage of DAP-5 by coxsackievirus B3 2A protease facilitates viral replication and enhances apoptosis by altering translation of IRES-containing genes. Cell Death Differ. 23: 828-840.

RESEARCH USE

For research use only, not for use in diagnostic procedures.