SANTA CRUZ BIOTECHNOLOGY, INC.

AMID siRNA (h): sc-72339

BACKGROUND

AMID (apoptosis-inducing factor (AIF)-like mitochondrion-associated inducer of death), also called p53-responsive gene 3 (PRG3), is a member of the FAD-dependent oxidoreductase family. AMID is a caspase independent pro-apoptotic flavoprotein with NAD(P)H oxidase activity localizing to the cytosol and associated with the outer mitochondrial membrane. AMID shares significant homology with AIF and NADH-oxidoreductases. It is expressed in most normal tissues and its expression is upregulated by p53. Two AMID isoforms exist due to alternative splicing. Isoform 1 is the full length protein and isoform 2 is missing amino acids 99-138. Isoform 2 also has an additional three amino acids inserted after residue 206. Overexpression of AMID leads to apoptosis.

REFERENCES

1. Wu, M., et al. 2002. AMID, an apoptosis-inducing factor-homologous mitochondrion-associated protein, induces caspase-independent apoptosis. J. Biol. Chem. 277: 25617-25623.
2. Lü, C.X., et al. 2003. Apoptosis-inducing factor and apoptosis. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao 35: 881-885.
3. Jan, Y., et al. 2004. A mitochondrial protein, Bit1, mediates apoptosis regulated by integrins and Groucho/TLE corepressors. Cell 116: 751-762.
4. Wu, M., et al. 2004. AMID is a p53-inducible gene downregulated in tumors. Oncogene 23: 6815-6819.
5. Marshall, K.R., et al. 2005. The human apoptosis-inducing protein AMID is an oxidoreductase with a modified flavin cofactor and DNA binding activity. J. Biol. Chem. 280: 30735-30740.
6. Hu, Q., et al. 2005. Endoplasmic reticulum mediated necrosis-like apoptosis of HeLa cells induced by Ca^{2+} oscillation. J. Biochem. Mol. Biol. 38: 709-716.
7. Li, W., et al. 2006. Yeast AMID homologue Ndi1p displays respiration-restricted apoptotic activity and is involved in chronological aging. Mol. Biol. Cell 17: 1802-1811.
8. Mei, J., et al. 2006. The p53-inducible apoptotic protein AMID is not required for normal development and tumor suppression. Oncogene 25: 849-856.

CHROMOSOMAL LOCATION

Genetic locus: AIFM2 (human) mapping to 10q22.1.

PRODUCT

AMID 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 AMID shRNA Plasmid (h): sc-72339-SH and AMID shRNA (h) Lentiviral Particles: sc-72339-V as alternate gene silencing products.

For independent verification of AMID (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-72339A, sc-72339B and sc-72339C.

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

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

AMID (B-6): sc-377120 is recommended as a control antibody for monitoring of AMID 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 AMID gene expression knockdown using RT-PCR Primer: AMID (h)-PR: sc-72339-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.