SANTA CRUZ BIOTECHNOLOGY, INC.

NIT1 siRNA (h): sc-78620

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Belonging to the large family of nonpeptidic C-N hydrolases, nitrilases are enzymes that cleave nitriles and organic amides, resulting in carboxlic acid and ammonia. NIT1 (nitrilase homolog 1) is a 327 amino acid protein that plays a role in cell growth and apoptosis. Loss of NIT1 expression leads to accelerated proliferation, increased cyclin D1 expression and resistance to DNA damage stress, whereas overexpression of NIT1 leads to caspase-dependent apoptosis. This evidence suggests that NIT1 functions as a tumor supressor. NIT1 is expressed in placenta, kidney, brain, liver, heart, pancreas and skeletal muscle where it is localized to both the cytoplasm and mitochondria. There are four isoforms of NIT1 that are produced as a result of alternative splicing events.

REFERENCES

1. Pekarsky, Y., et al. 1998. Nitrilase and Fhit homologs are encoded as fusion proteins in *Drosophila melanogaster* and *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 95: 8744-8749.
2. Online Mendelian Inheritance in Man, OMIM™. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 604618. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Vorwerk, S., et al. 2001. Enzymatic characterization of the recombinant *Arabidopsis thaliana* nitrilase subfamily encoded by the NIT2/NIT1/NIT3-gene cluster. Planta 212: 508-516.
4. O'Reilly, C. and Turner, P.D. 2003. The nitrilase family of CN hydrolysing enzymes—a comparative study. J. Appl. Microbiol. 95: 1161-1174.
5. Cutler, S.R. and Somerville, C.R. 2005. Imaging plant cell death: GFP-NIT1 aggregation marks an early step of wound and herbicide induced cell death. BMC Plant Biol. 5: 4.
6. Semba, S., et al. 2006. Biological functions of mammalian NIT1, the counterpart of the invertebrate NitFhit Rosetta stone protein, a possible tumor suppressor. J. Biol. Chem. 281: 28244-28253.
7. Barglow, K.T., et al. 2008. Functional proteomic and structural insights into molecular recognition in the nitrilase family enzymes. Biochemistry 47: 13514-13523.

CHROMOSOMAL LOCATION

Genetic locus: NIT1 (human) mapping to 1q23.3.

PRODUCT

NIT1 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 NIT1 shRNA Plasmid (h): sc-78620-SH and NIT1 shRNA (h) Lentiviral Particles: sc-78620-V as alternate gene silencing products.

For independent verification of NIT1 (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-78620A, sc-78620B and sc-78620C.

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

NIT1 siRNA (h) is recommended for the inhibition of NIT1 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

NIT1 (D-7): sc-515566 is recommended as a control antibody for monitoring of NIT1 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 NIT1 gene expression knockdown using RT-PCR Primer: NIT1 (h)-PR: sc-78620-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.