SANTA CRUZ BIOTECHNOLOGY, INC.

NT5C2 siRNA (h): sc-90370

BACKGROUND

Nucleotidases are hydrolytic enzymes that catalyze the hydrolysis of nucleotides into phosphates and nucleosides. NT5C2 (5'-nucleotidase, cytosolic II), also known as NT5B, NT5CP or PNT5, is a 561 amino acid protein that has an essential role in the maintenance of purine/pyrimidine nucleotides. NT5C2 contains a phosphotransferase active site that catalyzes the dephosphorylation of 6-hydroxypurine nucleoside 5'-monophosphates. NT5C2 is allosterically activated by ATP and various other compounds. In addition, NT5C2 regulates the level of inosine monophosphate (IMP) and guanosine monophosphate (GMP) pools within cells via hydrolysis. NT5C2 is structurally similar to enzymes of the haloacid dehalogenase (HAD) superfamily. Members of this superfamily function as hydrolases, converting haloacid to hydroxy acid and a halide. NT5C2 is localized to the cytoplasmic matrix of cells. Defects in the gene that encodes NT5C2 may cause increased uric acid concentrations in cells, which can lead to gout and/or hyperuricemia.

REFERENCES

1. Oka, J., et al. 1994. Molecular cloning of human cytosolic purine 5'-nucleotidase. Biochem. Biophys. Res. Commun. 205: 917-922.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 600417. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Galmarini, C.M., et al. 2005. The prognostic value of cN-II and cN-III enzymes in adult acute myeloid leukemia. Haematologica 90: 1699-1701.
4. Sève, P., et al. 2005. cN-II expression predicts survival in patients receiving gemcitabine for advanced non-small cell lung cancer. Lung Cancer 49: 363-370.
5. Jordheim, L.P., et al. 2006. F-ara-AMP is a substrate of cytoplasmic 5'-nucleotidase II (cN-II): HPLC and NMR studies of enzymatic dephosphorylation. Nucleosides Nucleotides Nucleic Acids 25: 289-297.
6. Galmarini, C.M. 2007. What does overexpression of cN-II enzyme signify in haematological malignancies? Leuk. Res. 31: 1325-1326.
7. Walldén, K., et al. 2007. Crystal structure of human cytosolic 5'-nucleotidase II: insights into allosteric regulation and substrate recognition. J. Biol. Chem. 282: 17828-17836.

CHROMOSOMAL LOCATION

Genetic locus: NT5C2 (human) mapping to 10q24.32.

PRODUCT

NT5C2 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 NT5C2 shRNA Plasmid (h): sc-90370-SH and NT5C2 shRNA (h) Lentiviral Particles: sc-90370-V as alternate gene silencing products.

For independent verification of NT5C2 (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-90370A, sc-90370B and sc-90370C.

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

NT5C2 siRNA (h) is recommended for the inhibition of NT5C2 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

NT5C2 (FF-12): sc-82000 is recommended as a control antibody for monitoring of NT5C2 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 NT5C2 gene expression knockdown using RT-PCR Primer: NT5C2 (h)-PR: sc-90370-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.