

NT5C3 siRNA (h): sc-89592

BACKGROUND

Nucleotidases are hydrolytic enzymes that catalyze the hydrolysis of nucleotides into phosphates and nucleosides. NT5C3 (5'-nucleotidase, cytosolic III), also known as P5N1 or UMPH1, is a 336 amino acid protein that exists as multiple alternatively spliced isoforms which localize to either the cytoplasm or the endoplasmic reticulum. Expressed in an isoform-specific manner in lymphocytes and reticulocytes, NT5C3 belongs to the pyrimidine 5'-nucleotidase family and exists as a monomer which acts as both a nucleotidase and a phosphotransferase, effectively catalyzing the conversion of a 5'-ribonucleotide to a ribonucleoside and a free phosphate. Defects in the gene encoding NT5C3 are the cause of P5N deficiency, an autosomal recessive disorder that is associated with hemolytic anemia and is characterized by lead poisoning and learning difficulties.

REFERENCES

1. Amici, A., et al. 1994. Homogeneous pyrimidine nucleotidase from human erythrocytes: enzymic and molecular properties. *Biochem. J.* 304: 987-992.
2. Amici, A., et al. 2000. Human erythrocyte pyrimidine 5-nucleotidase, PN-I, is identical to p36, a protein associated to lupus inclusion formation in response to α -interferon. *Blood* 96: 1596-1598.
3. Balta, G., et al. 2003. Molecular characterization of Turkish patients with pyrimidine 5' nucleotidase-I deficiency. *Blood* 102: 1900-1903.
4. Rees, D.C., et al. 2003. Pyrimidine 5' nucleotidase deficiency. *Br. J. Haematol.* 120: 375-383.
5. Bianchi, P., et al. 2003. Molecular characterization of six unrelated Italian patients affected by pyrimidine 5'-nucleotidase deficiency. *Br. J. Haematol.* 122: 847-851.
6. Online Mendelian Inheritance in Man, OMIM™. 2004. Johns Hopkins University, Baltimore, MD. MIM Number: 606224. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
7. Chiarelli, L.R., et al. 2005. Functional analysis of pyrimidine 5'-nucleotidase mutants causing nonspherocytic hemolytic anemia. *Blood* 105: 3340-3345.
8. Chondrogianni, N. and Gonos, E.S. 2007. Overexpression of hUMP1/POMP proteasome accessory protein enhances proteasome-mediated antioxidant defence. *Exp. Gerontol.* 42: 899-903.

CHROMOSOMAL LOCATION

Genetic locus: NT5C3A (human) mapping to 7p14.3.

PRODUCT

NT5C3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NT5C3 shRNA Plasmid (h): sc-89592-SH and NT5C3 shRNA (h) Lentiviral Particles: sc-89592-V as alternate gene silencing products.

For independent verification of NT5C3 (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-89592A, sc-89592B and sc-89592C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NT5C3 siRNA (h) is recommended for the inhibition of NT5C3 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 μ M in 66 μ l. 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

NT5C3 (E-8): sc-390782 is recommended as a control antibody for monitoring of NT5C3 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 NT5C3 gene expression knockdown using RT-PCR Primer: NT5C3 (h)-PR: sc-89592-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Noss, E.H., et al. 2015. Evidence for cadherin-11 cleavage in the synovium and partial characterization of its mechanism. *Arthritis Res. Ther.* 17: 126.

RESEARCH USE

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