

NT5C3L siRNA (h): sc-93563

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. NT5C3L (5'-nucleotidase, cytosolic III-like), also known as cN-III-like protein, is a 292 amino acid cytoplasmic protein belonging to the pyrimidine 5'-nucleotidase family that also functions as a nucleotidase and a phosphotransferase.

REFERENCES

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- Rees, D.C., et al. 2003. Pyrimidine 5' nucleotidase deficiency. *Br. J. Haematol.* 120: 375-383.
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CHROMOSOMAL LOCATION

Genetic locus: NT5C3B (human) mapping to 17q21.2.

PRODUCT

NT5C3L 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 NT5C3L shRNA Plasmid (h): sc-93563-SH and NT5C3L shRNA (h) Lentiviral Particles: sc-93563-V as alternate gene silencing products.

For independent verification of NT5C3L (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-93563A, sc-93563B and sc-93563C.

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

NT5C3L siRNA (h) is recommended for the inhibition of NT5C3L 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

NT5C3L (A-9): sc-398604 is recommended as a control antibody for monitoring of NT5C3L 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 NT5C3L gene expression knockdown using RT-PCR Primer: NT5C3L (h)-PR: sc-93563-PR (20 μ l). 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.