

NT5C1A shRNA (m) Lentiviral Particles: sc-150079-V

BACKGROUND

NT5C1A (5'-nucleotidase cytosolic 1A) is a 365 amino acid protein belonging to the 5'-nucleotidase type 3 family. Activated by ADP, NT5C1A uses magnesium and water to dephosphorylate the 5' and 2'(3')-phosphates of deoxyribonucleotides to produce a ribonucleoside and a phosphate. Localized to the cytoplasm, NT5C1A has broad substrate specificity. NT5C1A also assists in the regulation of adenosine levels in heart during ischemia and hypoxia. The gene that encodes NT5C1A maps to chromosome four, a chromosome containing nearly 900 genes, representing approximately 6% of the human genome. Chromosome 4 reportedly contains the largest gene deserts (regions of the genome with no protein encoding genes) and has one of the two lowest recombination frequencies of the human chromosomes.

REFERENCES

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2. Hillier, L.W., et al. 2005. Generation and annotation of the DNA sequences of human chromosomes 2 and 4. *Nature* 434: 724-731.
3. Ipata, P.L. and Tozzi, M.G. 2006. Recent advances in structure and function of cytosolic IMP-GMP specific 5'-nucleotidase II (cN-II). *Purinergic Signal*. 2: 669-675.
4. Lechward, K. and Tkacz-Stachowska, K. 2009. Expression of cytosolic 5' nucleotidase does not correlate with expression of oxidative metabolism marker: myoglobine in human skeletal muscles. *Acta Biochim. Biophys. Sin.* 41: 280-284.
5. Meijer, P., et al. 2009. Rosuvastatin increases extracellular adenosine formation in humans *in vivo*: a new perspective on cardiovascular protection. *Arterioscler. Thromb. Vasc. Biol.* 29: 963-968.

CHROMOSOMAL LOCATION

Genetic locus: *Nt5c1a* (mouse) mapping to 4 D2.2.

PRODUCT

NT5C1A shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see NT5C1A siRNA (m): sc-150079 and NT5C1A shRNA Plasmid (m): sc-150079-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

APPLICATIONS

NT5C1A shRNA (m) Lentiviral Particles is recommended for the inhibition of NT5C1A expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

NT5C1A (C-9): sc-377244 is recommended as a control antibody for monitoring of NT5C1A 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 NT5C1A gene expression knockdown using RT-PCR Primer: NT5C1A (m)-PR: sc-150079-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.