SANTA CRUZ BIOTECHNOLOGY, INC.

NT5C1A siRNA (h): sc-78565

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

1. Onishi, M., Yasunaga, T., Tanaka, H., Nishimune, Y. and Nozaki, M. 2004. Gene structure and evolution of testicular haploid germ cell-specific genes, Oxct2a and Oxct2b. Genomics 83: 647-657.
2. Hillier, L.W., Graves, T.A., Fulton, R.S., Fulton, L.A., Pepin, K.H., Minx, P., Wagner-McPherson, C., Layman, D., Wylie, K., Sekhon, M., Becker, M.C., Fewell, G.A., Delehaunty, K.D., Miner, T.L., Nash, W.E., Kremitzki, C., Oddy, L., Du, H., Sun, H., Bradshaw-Cordum, H,. Ali, J., Carter, J., Cordes, M., 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., Oyen, W.J., Dekker, D., van den Broek, P.H., Wouters, C.W., Boerman, O.C., Scheffer, G.J., Smits, P. and Rongen, G.A. 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 (human) mapping to 1p34.2.

PRODUCT

NT5C1A 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 NT5C1A shRNA Plasmid (h): sc-78565-SH and NT5C1A shRNA (h) Lentiviral Particles: sc-78565-V as alternate gene silencing products.

For independent verification of NT5C1A (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-78565A, sc-78565B and sc-78565C.

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

NT5C1A siRNA (h) is recommended for the inhibition of NT5C1A 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

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 (h)-PR: sc-78565-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.