SANTA CRUZ BIOTECHNOLOGY, INC.

CMAH siRNA (m): sc-142408

BACKGROUND

The sialic acids are a family of acidic sugars typically found in the outer portion of the cell surface and in secreted glycoconjugates of all vertebrates. Cell membrane sialic acid is involved in cell-cell and cell-pathogen interactions and in binding of cells to the extracellular matrix. The 2 most common forms of sialic acid found in mammalian cells are N-acetylneuraminic acid (Neu5Ac) and its hydroxylated derivative, N-glycolylneuraminic acid (Neu5Gc). CMAH (cytidine monophospho-N-acetylneuraminic acid hydroxylase), also known as CMP-Neu5Ac hydroxylase or CMP-N-acetylneuraminate monooxygenase, is a 577 amino acid cytoplasmic protein that is expressed in all tissues, except in brain. Belonging to the CMP-Neu5Ac hydroxylase family, CMAH catalyzes the conversion of CMP-Neu5Ac into its hydroxylated derivative CMP-Neu5Gc, a sialic acid abundantly expressed at the surface of many cells. CMAH exists as two isoforms due to alternative splicing events. Isoform 2 is expressed in the endoplasmic reticulum.

REFERENCES

1. Kawano, T., et al. 1995. Molecular cloning of cytidine monophospho-N-acetylneuraminic acid hydroxylase. Regulation of species- and tissue-specific expression of N-glycolylneuraminic acid. J. Biol. Chem. 270: 16458-16463.
2. Muchmore, E.A., et al. 1998. A structural difference between the cell surfaces of humans and the great apes. Am. J. Phys. Anthropol. 107: 187-198.
3. Online Mendelian Inheritance in Man, OMIM™. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 603209. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Chou, H.H., et al. 2002. Inactivation of CMP-N-acetylneuraminic acid hydroxylase occurred prior to brain expansion during human evolution. Proc. Natl. Acad. Sci. USA 99: 11736-11741.
5. Bighignoli, B., et al. 2007. Cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH) mutations associated with the domestic cat AB blood group. BMC Genet. 8: 27.

CHROMOSOMAL LOCATION

Genetic locus: Cmah (mouse) mapping to 13 A3.1.

PRODUCT

CMAH siRNA (m) 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 CMAH shRNA Plasmid (m): sc-142408-SH and CMAH shRNA (m) Lentiviral Particles: sc-142408-V as alternate gene silencing products.

For independent verification of CMAH (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-142408A, sc-142408B and sc-142408C.

PROTOCOLS

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

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

CMAH siRNA (m) is recommended for the inhibition of CMAH expression in mouse 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

CMAH (E-7): sc-365023 is recommended as a control antibody for monitoring of CMAH 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 CMAH gene expression knockdown using RT-PCR Primer: CMAH (m)-PR: sc-142408-PR (20 µl, 563 bp). 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.