SANTA CRUZ BIOTECHNOLOGY, INC.

PCMT1 siRNA (h): sc-95544

BACKGROUND

PCMT1 (protein-L-isoaspartate (D-aspartate) O-methyltransferase), also known as PIMT, is a member of the L-isoaspartyl/D-aspartyl protein methyltransferase family and is highly expressed in brain. Functioning as a monomer, PCMT localizes to the cytoplasm and participates in the degradation and/or repair of damaged proteins. More specifically, PCMT1 recognizes isomerized Asp or Asn residues in peptides and proteins and catalyzes the conversion of abnormal L-isoaspartyl and D-aspartyl residues to methyl esters that may then spontaneously hydrolyze to re-form normal aspartyl residues. In congruence with this reaction, PCMT1 converts the methyl donor S-adenosylmethionine (AdoMet) to S-andenosylhomocysteine (AdoHcy). In mice lacking PCMT1, damaged proteins accumulate in a variety of tissues and the ratio between AdoMet and AdoHcy is increased in brain tissue. The phenotypic result is progressive epilepsy and death at an early age.

REFERENCES

1. MacLaren, D.C., et al. 1992. The L-isoaspartyl/D-aspartyl protein methyltransferase gene (PCMT1) maps to human chromosome 6q22.3-6q24 and the syntenic region of mouse chromosome 10. Genomics 14: 852-856.
2. MacLaren, D.C., et al. 1992. Alternative splicing of the human isoaspartyl protein carboxyl methyltransferase RNA leads to the generation of a C-terminal-RDEL sequence in isozyme II. Biochem. Biophys. Res. Commun. 185: 277-283.
3. DeVry, C.G. and Clarke, S. 1999. Assignment of the protein L-isoaspartate (D-aspartate) O-methyltransferase gene (PCMT1) to human chromosome bands 6q24→q25 with radiation hybrid mapping. Cytogenet. Cell Genet. 84: 130-131.
4. DeVry, C.G. and Clarke, S. 1999. Polymorphic forms of the protein L-isoaspartate (D-aspartate) O-methyltransferase involved in the repair of age-damaged proteins. J. Hum. Genet. 44: 275-288.
5. Farrar, C. and Clarke, S. 2002. Altered levels of S-adenosylmethionine and S-adenosylhomocysteine in the brains of L-isoaspartyl (D-Aspartyl) O-methyltransferase-deficient mice. J. Biol. Chem. 277: 27856-27863.

CHROMOSOMAL LOCATION

Genetic locus: PCMT1 (human) mapping to 6q25.1.

PRODUCT

PCMT1 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 PCMT1 shRNA Plasmid (h): sc-95544-SH and PCMT1 shRNA (h) Lentiviral Particles: sc-95544-V as alternate gene silencing products.

For independent verification of PCMT1 (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-95544A, sc-95544B and sc-95544C.

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

PCMT1 siRNA (h) is recommended for the inhibition of PCMT1 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

PCMT1 (D-E7): sc-100977 is recommended as a control antibody for monitoring of PCMT1 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 PCMT1 gene expression knockdown using RT-PCR Primer: PCMT1 (h)-PR: sc-95544-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.