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PCMT1 siRNA (m): sc-152112

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, PCMT1 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-adenosylhomocysteine (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., et al. 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., et al. 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., et al. 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 (mouse) mapping to 10 A1.

PRODUCT

PCMT1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PCMT1 shRNA Plasmid (m): sc-152112-SH and PCMT1 shRNA (m) Lentiviral Particles: sc-152112-V as alternate gene silencing products.

For independent verification of PCMT1 (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-152112A, sc-152112B and sc-152112C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

PCMT1 siRNA (m) is recommended for the inhibition of PCMT1 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 μ M in 60 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

PCMT1 (C-15): sc-168915 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PCMT1 gene expression knockdown using RT-PCR Primer: PCMT1 (m)-PR: sc-152112-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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