

PCMTD2 siRNA (m): sc-152114

BACKGROUND

PCMTD2 (protein-L-isoaspartate O-methyltransferase domain-containing protein 1) is a 361 amino acid cytoplasmic protein that is a member of the L-isoaspartyl/D-aspartyl protein methyltransferase family. Members of this family participate in the degradation and/or repair of damaged proteins by specifically recognizing isomerized Asp or Asn residues in peptides and proteins. PCMTD2 is related to PCMT1, which 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 mice lacking PCMT1, damaged proteins accumulate in a variety of tissues and the phenotypic result is progressive epilepsy and death at an early age. There are three isoforms of PCMTD2 that are produced as a result of alternative splicing events.

REFERENCES

1. MacLaren, D.C., O'Connor, C.M., Xia, Y.R., Mehrabian, M., Klisak, I., Sparkes, R.S., Clarke, S. and Lusk, A.J. 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. Galletti, P., Ingrosso, D., Manna, C., Clemente, G. and Zappia, V. 1995. Protein damage and methylation-mediated repair in the erythrocyte. *Biochem. J.* 306: 313-325.
3. Yamamoto, A., Takagi, H., Kitamura, D., Tatsuoka, H., Nakano, H., Kawano, H., Kuroyanagi, H., Yahagi, Y., Kobayashi, S., Koizumi, K., Sakai, T., Saito, K., Chiba, T., Kawamura, K., Suzuki, K., Watanabe, T., Mori, H., et al. 1998. Deficiency in protein L-isoaspartyl methyltransferase results in a fatal progressive epilepsy. *J. Neurosci.* 18: 2063-2074.
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.
6. Clarke, S. 2003. Aging as war between chemical and biochemical processes: protein methylation and the recognition of age-damaged proteins for repair. *Ageing Res. Rev.* 2: 263-285.
7. Wagner, A.M., Cloos, P., Bergholdt, R., Boissy, P., Andersen, T.L., Henriksen, D.B., Christiansen, C., Christgau, S., Pociot, F. and Nerup, J. 2007. Post-translational protein modifications in type 1 diabetes: a role for the repair enzyme protein-L-isoaspartate (D-aspartate) O-methyltransferase? *Diabetologia* 50: 676-681.
8. Alfaro, J.F., Gillies, L.A., Sun, H.G., Dai, S., Zang, T., Klaene, J.J., Kim, B.J., Lowenson, J.D., Clarke, S.G., Karger, B.L. and Zhou, Z.S. 2008. Chemo-enzymatic detection of protein isoaspartate using protein isoaspartate methyltransferase and hydrazine trapping. *Anal. Chem.* 80: 3882-3889.
9. Pyun, J.A., Kang, H., Lee, S.K., Kim, M.H. and Kwack, K. 2009. Association between polymorphisms in the protein L-isoaspartate (D-aspartate) O-methyltransferase gene and premature ovarian failure. *Fertil. Steril.* 91: 1362-1365.

CHROMOSOMAL LOCATION

Genetic locus: Pcmt2 (mouse) mapping to 2 H4.

PRODUCT

PCMTD2 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 PCMTD2 shRNA Plasmid (m): sc-152114-SH and PCMTD2 shRNA (m) Lentiviral Particles: sc-152114-V as alternate gene silencing products.

For independent verification of PCMTD2 (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-152114A, sc-152114B and sc-152114C.

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

PCMTD2 siRNA (m) is recommended for the inhibition of PCMTD2 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 66 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PCMTD2 gene expression knockdown using RT-PCR Primer: PCMTD2 (m)-PR: sc-152114-PR (20 μ l). 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.