SANTA CRUZ BIOTECHNOLOGY, INC.

PMCA4b siRNA (h): sc-36279

BACKGROUND

The plasma membrane Ca^{2+}-pumping ATPase (PMCA) mRNAs are encoded on four genes designated PMCA1-4. The PMCA genes undergo alternative splicing to yield "b" splice forms, which contain PDZ interaction domains and interact promiscuously but also specifically with different members of the PSD95 family. PMCA4b is the major PMCA expressed in developing mammary tissue. During lactation, PMCA1b expression increases while PMCA4b expression decreases, indicating that PMCAs play a critical role in maintaining cellular Ca^{2+} homeostasis. In addition, human PMCA4b may have an important role in regulating intracellular Ca^{2+} in the apoptotic cell. PMCA4b is cleaved at Asp 1,080 by caspase-3 to produce a single fragment that is fully active, responding much faster to an increase in Ca^{2+} than the autoinhibited form. PMCA4b also plays an essential role in maintaining low cytosolic Ca^{2+} in resting platelets. Specifically, PMCA4b is phosphorylated on Tyr 1176 by pp60 (src).

REFERENCES

1. Brandt, P., et al. 1992. Analysis of the tissue-specific distribution of mRNAs encoding the plasma membrane calcium-pumping ATPases and characterization of an alternately spliced form of PMCA4 at the cDNA and gemonic levels. J. Biol. Chem. 267: 4376-4385.
2. Dean, W.L., et al. 1997. Regulation of platelet plasma membrane Ca^{2+}-ATPase by cAMP-dependent and tyrosine phosphorylation. J. Biol. Chem. 272: 15113-15119.
3. Reinhardt, T.A., et al. 1999. Ca^{2+}-ATPases and their expression in the mammary gland of pregnant and lactating rats. Am. J. Physiol. 276: 796-802.
4. Reinhardt, T.A., et al. 2000. Ca^{2+}-ATPase protein expression in mammary tissue. Am. J. Physiol. Cell Physiol. 279: 1595-1602.
5. Zabe, M., et al. 2001. Plasma membrane Ca^{2+}-ATPase associates with the cytoskeleton in activated platelets through a PDZ-binding domain. J. Biol. Chem. 276: 14704-14709.
6. DeMarco, S.J., et al. 2001. Plasma membrane Ca^{2+}-atpase isoforms 2b and 4b interact promiscuously and selectively with members of the membrane-associated guanylate kinase family of PDZ (PDS95/Dlg/ZO-1) domain-containing proteins. J. Biol. Chem. 276: 21594-25600.

CHROMOSOMAL LOCATION

Genetic locus: ATP2B4 (human) mapping to 1q32.1.

PRODUCT

PMCA4b 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 PMCA4b shRNA Plasmid (h): sc-36279-SH and PMCA4b shRNA (h) Lentiviral Particles: sc-36279-V as alternate gene silencing products.

For independent verification of PMCA4b (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-36279A, sc-36279B and sc-36279C.

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

PMCA4b siRNA (h) is recommended for the inhibition of PMCA4b 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.

RECOMMENDED SECONDARY REAGENTS

PMCA4b (JA3): sc-20027 is recommended as a control antibody for monitoring of PMCA4b 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 PMCA4b gene expression knockdown using RT-PCR Primer: PMCA4b (h)-PR: sc-36279-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.