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PMCA1 siRNA (m): sc-42597

BACKGROUND

Plasma membrane-type Ca^{2+} -ATPases (PMCA) mediate the export of bivalent calcium ions from eukaryotic cells. As members of the P class of ion-motive ATPases, PMCA are a functionally diverse group of proteins that are derived from alternatively spliced transcripts originating from at least four distinct genes. The expression of different PMCA isoforms and splice variants is regulated in a developmental, tissue- and cell type-specific manner, and with respect to the physiological needs of specific cell and tissue types. Spatial and temporal rates of resting intracellular Ca^{2+} concentrations and Ca^{2+} signaling in eukaryotic cells are dependent on the array of PMCA isoforms that are expressed in concert with the rate of Ca^{2+} export. PMCA1 (ATP2B1) is a ubiquitously expressed form of the PMCA calcium exporter family.

REFERENCES

1. Greeb, J. and Shull, G.E. 1989. Molecular cloning of a third isoform of the calmodulin-sensitive plasma membrane Ca^{2+} -transporting ATPase that is expressed predominantly in brain and skeletal muscle. *J. Biol. Chem.* 264: 18569-18576.
2. Olson, S., Wang, M.G., Carafoli, E., Strehler, E.E. and McBride, O.W. 1991. Localization of two genes encoding plasma membrane Ca^{2+} -transporting ATPases to human chromosomes 1q25-32 and 12q21-23. *Genomics* 9: 629-641.
3. Fresu, L., Dehpour, A., Genazzani, A.A., Carafoli, E. and Guerini, D. 1999. Plasma membrane calcium ATPase isoforms in astrocytes. *Glia* 28: 150-155.
4. Caride, A.J., Filoteo, A.G., Penheiter, A.R., Pászty, K., Enyedi, A. and Penniston, J.T. 2001. Delayed activation of the plasma membrane calcium pump by a sudden increase in Ca^{2+} : fast pumps reside in fast cells. *Cell Calcium* 30: 49-57.
5. Strehler, E.E. and Zacharias, D.A. 2001. Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. *Physiol. Rev.* 81: 21-50.

CHROMOSOMAL LOCATION

Genetic locus: *Atp2b1* (mouse) mapping to 10 C3.

PRODUCT

PMCA1 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 PMCA1 shRNA Plasmid (m): sc-42597-SH and PMCA1 shRNA (m) Lentiviral Particles: sc-42597-V as alternate gene silencing products.

For independent verification of PMCA1 (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-42597A, sc-42597B and sc-42597C.

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

PMCA1 siRNA (m) is recommended for the inhibition of PMCA1 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

PMCA1 (F-10): sc-398413 is recommended as a control antibody for monitoring of PMCA1 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 PMCA1 gene expression knockdown using RT-PCR Primer: PMCA1 (m)-PR: sc-42597-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

RESEARCH USE

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