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PMCA4 siRNA (h): sc-42602

BACKGROUND

Plasma membrane-type Ca²⁺-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 four distinct genes, PMCA1, 2, 3 and 4. 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 Ca²⁺ concentrations and Ca²⁺ signaling in eukaryotic cells are dependent on the array of PMCA isoforms that are expressed in concert with the rate of Ca²⁺ export. PMCA3 expression is confined to brain and skeletal muscle.

REFERENCES

- Olson, S., et al. 1991. Localization of two genes encoding plasma membrane Ca²⁺-transporting ATPases to human chromosomes 1q25-32 and 12q21-23. *Genomics* 9: 629-641.
- Eakin, T.J., et al. 1995. Localization of the plasma membrane Ca²⁺-ATPase isoform PMCA3 in rat cerebellum, choroid plexus and hippocampus. *Brain Res. Mol. Brain Res.* 29: 71-80.
- Garcia, M.L., et al. 1999. Plasma membrane calcium ATPases as critical regulators of calcium homeostasis during neuronal cell function. *Front. Biosci.* 4: 869-882.
- Fresu, L., et al. 1999. Plasma membrane calcium ATPase isoforms in astrocytes. *Glia* 28: 150-155.
- Strehler, E.E., et al. 2001. Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. *Physiol. Rev.* 81: 21-50.
- Caride, A.J., et al. 2001. Delayed activation of the plasma membrane calcium pump by a sudden increase in Ca²⁺: fast pumps reside in fast cells. *Cell Calcium* 30: 49-57.

CHROMOSOMAL LOCATION

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

PRODUCT

PMCA4 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PMCA4 shRNA Plasmid (h): sc-42602-SH and PMCA4 shRNA (h) Lentiviral Particles: sc-42602-V as alternate gene silencing products.

For independent verification of PMCA4 (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-42602A, sc-42602B and sc-42602C.

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 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

PMCA4 siRNA (h) is recommended for the inhibition of PMCA4 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 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.

GENE EXPRESSION MONITORING

PMCA (F-3): sc-271917 is recommended as a control antibody for monitoring of PMCA4 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 PMCA4 gene expression knockdown using RT-PCR Primer: PMCA4 (h)-PR: sc-42602-PR (20 μ l, 443 bp). 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.