

PMR1 siRNA (h): sc-36285

BACKGROUND

The *Saccharomyces cerevisiae* protein, PMR1, encodes P-type calcium transport ATPase, which localizes to the Golgi and regulates the intracellular transport of calcium and manganese. The human homologue, ATP2C1 (also designated SPLA in rat), also regulates the transport of calcium in the Golgi complex and is related to other P-type ATPases family members, such as the sarcoendoplasmic calcium ATPase (SERCA) and the plasma membrane calcium ATPase (PCMA). PMR1 is a transmembrane protein that exists as 2 splice variants, which vary by 20 amino acids. PMR1 is mutated in Hailey-Hailey disease (HHD), which is an autosomal dominant disorder that is characterized by blisters and erosions of the skin. These findings provide further evidence that PMR1 plays a key role in maintaining the integrity of the epidermis by controlling intracellular calcium signaling.

REFERENCES

1. Gunteski-Hamblin, A.M., et al. 1992. Molecular cloning and tissue distribution of alternatively spliced mRNAs encoding possible mammalian homologues of the yeast secretory pathway calcium pump. *Biochemistry* 31: 7600-7608.
2. Sorin, A., et al. 1997. PMR1, a Ca^{2+} -ATPase in yeast Golgi, has properties distinct from sarco/endoplasmic reticulum and plasma membrane calcium pumps. *J. Biol. Chem.* 272: 9895-9901.
3. Wei, Y., et al. 1999. An N-terminal EF hand-like motif modulates ion transport by PMR1, the yeast Golgi $\text{Ca}^{2+}/\text{Mn}^{2+}$ -ATPase. *Biochemistry* 38: 14534-14541.
4. Sudbrak, R., et al. 2000. Hailey-Hailey disease is caused by mutations in ATP2C1 encoding a novel Ca^{2+} pump. *Hum. Mol. Genet.* 9: 1131-1140.
5. Hu, Z., et al. 2000. Mutations in ATP2C1, encoding a calcium pump, cause Hailey-Hailey disease. *Nat. Genet.* 24: 61-65.

CHROMOSOMAL LOCATION

Genetic locus: ATP2C1 (human) mapping to 3q22.1.

PRODUCT

PMR1 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 PMR1 shRNA Plasmid (h): sc-36285-SH and PMR1 shRNA (h) Lentiviral Particles: sc-36285-V as alternate gene silencing products.

For independent verification of PMR1 (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-36285A, sc-36285B and sc-36285C.

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

PMR1 siRNA (h) is recommended for the inhibition of PMR1 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.

GENE EXPRESSION MONITORING

PMR1 (G-9): sc-365375 is recommended as a control antibody for monitoring of PMR1 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 PMR1 gene expression knockdown using RT-PCR Primer: PMR1 (h)-PR: sc-36285-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.