SANTA CRUZ BIOTECHNOLOGY, INC.

phospholamban siRNA (h): sc-39143

BACKGROUND

The Sarco(endo)plasmic-reticulum (SER) regulatory protein, phospholamban (PLB), is a small, plasma membrane-associated phospho-protein found in the SER of cardiac, smooth and slow-twitch muscle. Believed to assemble into a pentamer, PLB regulates cardiac contractility and Ca^{2+} affinity for cardiac SER Ca^{2+} ATPase (SERCA2a). Non-phosphorylated PLB associates with SERCA2a, and inhibits Ca^{2+} reuptake into the SER. PLB activation occurs when key Serine/Threonine residues in PLB (SER-10, SER-16, Thr-17) are phosphorylated by numerous effectors, which include PKC, PKA, PKG, and CaM kinase. Phosphorylation of PLB causes dissociation from SERCA2a and a subsequent increase in the rate of Ca^{2+} reuptake into the SER, which accelerates ventricular relaxation.

REFERENCES

1. Koss, K.L. and Kranias, E.G. 1996. Phospholamban: a prominent regulator of myocardial contractility. Circ. Res. 79: 1059-1063.
2. Arkin, I.T., et al. 1997. Structural perspectives of phospholamban, a helical transmembrane pentamer. Annu. Rev. Biophys. Biomol. Struct. 26: 157-179.
3. Coyler, J. 1998. Phosphorylation states of phospholamban. Ann. N.Y. Acad. Sci. 853: 79-91.
4. Adams, P.D., et al. 1998. Models for the transmembrane region of the phospholamban pentamer: which is correct? Ann. N.Y. Acad. Sci. 853: 178-185.
5. Minamisawa, S., et al. 1999. Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. Cell 99: 313-322.
6. Zhai, J., et al. 2000. Cardiac-specific overexpression of a superinhibitory pentameric phospholamban mutant enhances inhibition of cardiac function *in vivo*. J. Biol. Chem. 275: 10538-10544.

CHROMOSOMAL LOCATION

Genetic locus: PLN (human) mapping to 6q22.31.

PRODUCT

phospholamban 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 phospholamban shRNA Plasmid (h): sc-39143-SH and phospholamban shRNA (h) Lentiviral Particles: sc-39143-V as alternate gene silencing products.

For independent verification of phospholamban (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-39143A, sc-39143B and sc-39143C.

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

phospholamban siRNA (h) is recommended for the inhibition of phospholamban 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

phospholamban (F-7): sc-393990 is recommended as a control antibody for monitoring of phospholamban 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 phospholamban gene expression knockdown using RT-PCR Primer: phospholamban (h)-PR: sc-39143-PR (20 µl, 489 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.