SANTA CRUZ BIOTECHNOLOGY, INC.

Junctophilin-1 siRNA (h): sc-72005

BACKGROUND

Junctophilins are components of the junctional complexes between plasma membranes and endoplasmic or sarcoplasmic reticulums present in all excitable cells. Junctophilins contain a cytoplasmic domain which binds to the plasma membrane, as well as an ER/SR membrane spanning hydrophobic C-terminal segment. The three subtypes in this family are Junctophilin-1, -2 and -3. Junctophilin-1 is predominantly expressed in skeletal muscle, but is also expressed at low levels in heart. Junctophilin-2 is expressed in heart and skeletal muscle. Mutant mice lacking the Jph2 gene exhibit embryonic lethality and possess cardiac myocytes that express abnormal calcium transients. Junctophilin-3 is expressed in brain. The JPH3 alternatively spliced exon 2A has been suggested as a site for CTG repeat expansion leading to a Huntington disease-like autosomal dominant disorder.

REFERENCES

1. Takeshima, H., et al. 2000. Junctophilins: a novel family of junctional membrane complex proteins. Mol. Cell 6: 11-22.
2. Margolis, R.L., et al. 2001. A disorder similar to Huntington's disease is associated with a novel CAG repeat expansion. Ann. Neurol. 50: 373-380.
3. Takeshima, H., et al. 2001. Junctophilins: molecular components contributing junctional membrane complexes between the cell-surface membrane and endoplasmic/sarcoplasmic reticulum. Clin. Calcium 11: 758-762.
4. Komazaki, S., et al. 2002. Deficiency of triad formation in developing skeletal muscle cells lacking junctophilin type 1. FEBS Lett. 524: 225-229.
5. Komazaki, S., et al. 2003. Abnormal junctional membrane structures in cardiac myocytes expressing ectopic junctophilin type 1. FEBS Lett. 542: 69-73.
6. Moriguchi, S., et al. 2006. Functional uncoupling between Ca^{2+} release and afterhyperpolarization in mutant hippocampal neurons lacking junctophilins. Proc. Natl. Acad. Sci. USA 103: 10811-10816.
7. Phimister, A.J., et al. 2007. Conformation-dependent stability of junctophilin 1 (JP1) and ryanodine receptor type 1 (RyR1) channel complex is mediated by their hyper-reactive thiols. J. Biol. Chem. 282: 8667-8677.

CHROMOSOMAL LOCATION

Genetic locus: JPH1 (human) mapping to 8q21.11.

PRODUCT

Junctophilin-1 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 Junctophilin-1 shRNA Plasmid (h): sc-72005-SH and Junctophilin-1 shRNA (h) Lentiviral Particles: sc-72005-V as alternate gene silencing products.

For independent verification of Junctophilin-1 (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-72005A, sc-72005B and sc-72005C.

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

Junctophilin-1 siRNA (h) is recommended for the inhibition of Junctophilin-1 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

Junctophilin-1 (2E6): sc-517084 is recommended as a control antibody for monitoring of Junctophilin-1 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 Junctophilin-1 gene expression knockdown using RT-PCR Primer: Junctophilin-1 (h)-PR: sc-72005-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.