SANTA CRUZ BIOTECHNOLOGY, INC.

RyR-1 siRNA (h): sc-36449

BACKGROUND

Dihydropyridine receptor (DHPR) and the sarcoplasmic reticulum ryanodine receptor (RyR) are two key components of the intracellular junctions, where depolarization of the surface membrane is converted into the release of Ca^{2+} from internal stores. The RyR family consists of RyR-1, RyR-2 and RyR-3, which are characterized respectively as skeletal muscle, cardiac and brain ryanodine receptors. RyR proteins are essential for calcium-dependent excitation. Cells that do not express RyR lack excitation-contraction coupling and exhibit a several-fold reduction in Ca^{2+} current density. RyR-1 is expressed in slow- and fast-twitch skeletal muscle. Activation of RyR-1 leads to the release of Ca^{2+} from the sarcoplasmic reticulum (SR) which, in turn leads to muscle contraction. Mutations in the gene for this protein can result in a variety of muscle diseases, including Brody disease, malignant hyperthermia, cardiomyopathy and central core disease.

REFERENCES

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2. Fan, H., Brandt, N.R., Peng, M., Schwartz, A. and Caswell, A.H. 1995. Binding sites of monoclonal antibodies and dihydropyridine receptor α1 subunit cytoplasmic II-III loop on skeletal muscle triadin fusion peptides. Biochemistry 34: 14893-14901.
3. Lu, X., Xu, L. and Meissner, G. 1995. Phosphorylation of dihydropyridine receptor II-III loop peptide regulates skeletal muscle calcium release channel function. Evidence for an essential role of the β-OH group of Ser687. J. Biol. Chem. 270: 18459-18464.
4. Powell, J.A., Petherbridge, L. and Flucher, B.E. 1996. Formation of triads without the dihydropyridine receptor α subunits in cell lines from dysgenic skeletal muscle. J. Cell Biol. 134: 375-387.
5. Flucher, B.E. and Franzini-Armstrong, C. 1996. Formation of junctions involved in excitation-contraction coupling in skeletal and cardiac muscle. Proc. Natl. Acad. Sci. USA 93: 8101-8106.

CHROMOSOMAL LOCATION

Genetic locus: RYR1 (human) mapping to 19q13.2.

PRODUCT

RyR-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 RyR-1 shRNA Plasmid (h): sc-36449-SH and RyR-1 shRNA (h) Lentiviral Particles: sc-36449-V as alternate gene silencing products.

For independent verification of RyR-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-36449A, sc-36449B and sc-36449C.

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

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

RyR (F-1): sc-376507 is recommended as a control antibody for monitoring of RyR-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 RyR-1 gene expression knockdown using RT-PCR Primer: RyR-1 (h)-PR: sc-36449-PR (20 µl, 568 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.