SANTA CRUZ BIOTECHNOLOGY, INC.

calsequestrin 2 siRNA (m): sc-43278

BACKGROUND

Calsequestrin (CS), also known as CSQ, is the major calcium-binding protein of cardiac and skeletal muscle whose function is to sequester calcium in the lumen of the sarcoplasmic reticulum (SR). In mammals, there are two forms of this protein, calsequestrin 1 and calsequestrin 2, which encode fast-twitch skeletal muscle and cardiac calsequestrin, respectively. The form of calsequestrin 1 known as Calmitin is located in the terminal cisternae luminal spaces of the SR of fast skeletal muscle cells. Calsequestrin 2 is present in the terminal cisternae luminal spaces of the SR in both cardiac and slow skeletal muscle cells. In addition, calsequestrin regulates the ryanodine receptor signalling (RyR) through triadin and junctin.

REFERENCES

1. Barker, P.A., et al. 1988. An improved method for the isolation of rat cardiac sarcoplasmic reticulum. Mol. Cell. Biochem. 84: 87-95.
2. Gunji, K., et al. 1999. A 63 kDa skeletal muscle protein associated with eye muscle inflammation in Graves' disease is identified as the calcium binding protein calsequestrin. Autoimmunity 29: 1-9.
3. Shutova, A.N., et al. 1999. Comparative characteristics of sarcoplasmic reticulum preparations from skeletal muscles of the ground squirrel *Spermophilus undulatus,* rats, and rabbits. Biochemistry 64: 1250-1257.
4. Cho, J.H., et al. 2000. Calsequestrin, a calcium sequestering protein localized at the sarcoplasmic reticulum, is not essential for body-wall muscle function in *Caenorhabditis elegans*. J. Cell Sci. 113: 3947-3958.
5. Nori, A., et al. 2000. Site-directed mutagenesis and deletion of three phosphorylation sites of calsequestrin of skeletal muscle sarcoplasmic reticulum. Effects on intracellular targeting. Exp. Cell Res. 260: 40-49.
6. Shin, D.W., et al. 2000. The asp-rich region at the carboxyl-terminus of calsequestrin binds to Ca^{2+} and interacts with triadin. FEBS Lett. 486: 178-182.
7. Nori, A., et al. 2001. Targeting of calsequestrin to the sarcoplasmic reticulum of skeletal muscle upon deletion of its glycosylation site. Exp. Cell Res. 265: 104-113.

CHROMOSOMAL LOCATION

Genetic locus: Casq2 (mouse) mapping to 3 F2.2.

PRODUCT

calsequestrin 2 siRNA (m) 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 calsequestrin 2 shRNA Plasmid (m): sc-43278-SH and calsequestrin 2 shRNA (m) Lentiviral Particles: sc-43278-V as alternate gene silencing products.

For independent verification of calsequestrin 2 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43278A, sc-43278B and sc-43278C.

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

calsequestrin 2 siRNA (m) is recommended for the inhibition of calsequestrin 2 expression in mouse 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

calsequestrin 2 (E-12): sc-390999 is recommended as a control antibody for monitoring of calsequestrin 2 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 calsequestrin 2 gene expression knockdown using RT-PCR Primer: calsequestrin 2 (m)-PR: sc-43278-PR (20 µl, 579 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.