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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

calsequestrin 1 shRNA (m) Lentiviral Particles: sc-43276-V

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. Calsequestrin 1, also known as Calmitin, is located in the terminal cisternae luminal spaces of the SR of fast skeletal muscle cells. Calsequestrin 2 is present in terminal cisternae luminal spaces of the SR of both cardiac and slow skeletal muscle cells. In addition, calsequestrin regulates the ryanodine receptor signaling (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.

CHROMOSOMAL LOCATION

Genetic locus: Casq1 (mouse) mapping to 1 H3.

PRODUCT

calsequestrin 1 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see calsequestrin 1 siRNA (m): sc-43276 and calsequestrin 1 shRNA Plasmid (m): sc-43276-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

calsequestrin 1 shRNA (m) Lentiviral Particles is recommended for the inhibition of calsequestrin 1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

calsequestrin 1 (D-10): sc-137080 is recommended as a control antibody for monitoring of calsequestrin 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor calsequestrin 1 gene expression knockdown using RT-PCR Primer: calsequestrin 1 (m)-PR: sc-43276-PR (20 µl, 619 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.