

SERCA1 siRNA (m): sc-36483

BACKGROUND

ATP dependent calcium pumps are responsible, in part, for the maintenance of low cytoplasmic free calcium concentrations. The ATP pumps that reside in intracellular organelles are encoded by a family of structurally related enzymes, termed the sarcoplasmic or endoplasmic reticulum calcium (SERCA) ATPases. The sarcoplasmic reticulum of striated muscle is a specialized intracellular membrane system that plays a critical role in the contraction and relaxation of muscle. The SERCAs mediate Ca^{2+} uptake into intracellular stores. SERCA-mediated Ca^{2+} uptake induces and maintains muscular relaxation. The SERCA1 gene is exclusively expressed in type II (fast) skeletal muscle. The SERCA2 gene is subject to tissue-dependent processing which is responsible for the generation of the SERCA2a muscle-specific form expressed in type I (slow) skeletal, cardiac and smooth muscle, and the SERCA2b isoform expressed in all cell types. The SERCA3 gene is not as well characterized and is found in non-muscle cells. SERCA2 plays an important part in regulating cardiac contractile function. SERCA3 is an isoform expressed in several cell types including platelets, lymphoid cells and mast cells. SERCA1, SERCA2 and SERCA3 all undergo alternative splicing.

REFERENCES

1. Aubier, M., et al. 1998. Calcium ATPase and respiratory muscle function. *Eur. Respir. J.* 11: 758-766.
2. Anger, M., et al. 1998. Cellular distribution of Ca^{2+} pumps and Ca^{2+} release channels in rat cardiac hypertrophy induced by aortic stenosis. *Circulation* 98: 2477-2486.
3. Loukianov, E., et al. 1998. Enhanced myocardial contractility and increased Ca^{2+} transport function in transgenic hearts expressing the fast-twitch skeletal muscle sarcoplasmic reticulum Ca^{2+} -ATPase. *Circ. Res.* 83: 889-897.
4. Bobe, R., et al. 1998. Expression of two isoforms of the third sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA3) in platelets. Possible recognition of the SERCA3b isoform by the PL/IM430 monoclonal antibody. *FEBS Lett.* 423: 259-264.
5. Poch, E., et al. 1998. Functional characterization of alternatively spliced human SERCA3 transcripts. *Am. J. Physiol., Cell Physiol.* 275: C1449-C1458.

CHROMOSOMAL LOCATION

Genetic locus: Atp2a1 (mouse) mapping to 7 F3.

PRODUCT

SERCA1 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 SERCA1 shRNA Plasmid (m): sc-36483-SH and SERCA1 shRNA (m) Lentiviral Particles: sc-36483-V as alternate gene silencing products.

For independent verification of SERCA1 (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-36483A, sc-36483B and sc-36483C.

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

SERCA1 siRNA (m) is recommended for the inhibition of SERCA1 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

SERCA1 (A-6): sc-515162 is recommended as a control antibody for monitoring of SERCA1 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-IgG λ BP-HRP: sc-516132 or m-IgG λ BP-HRP (Cruz Marker): sc-516132-CM (dilution range: 1:1000-1:10000), Cruz MarkerTM Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG λ BP-FITC: sc-516185 or m-IgG λ BP-PE: sc-516186 (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 SERCA1 gene expression knockdown using RT-PCR Primer: SERCA1 (m)-PR: sc-36483-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

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.