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Sarcospan siRNA (h): sc-43426

BACKGROUND

The dystrophin-glycoprotein complex (DGC) is a multisubunit protein complex that spans the sarcolemma and forms a link between the subsarcolemmal cytoskeleton and the extracellular matrix. Defects in components of the DGC cause muscular dystrophy, indicating that the DGC plays important roles in muscular function and viability. Sarcospan (also designated K-Ras oncogene-associated protein and Kirstein-Ras-associated protein), a member of this complex, contains four transmembrane spanning helices with intracellular N- and C-terminal domains. The expression of Sarcospan is reduced in muscle from patients with Duchenne muscular dystrophy. Sarcospan mRNAs are expressed in a range of cell lines, tumors, and normal tissue, with very high expression levels in muscle. Two isoforms of Sarcospan, SPN1 and SPN2, are produced by alternative splicing. SPN1 is expressed in heart and skeletal muscle, whereas SPN2 is expressed in heart, skeletal muscle, thymus, prostate, testis, ovary, small intestine, colon and spleen. The sarcoglycan complex in striated muscle is a heterotetrameric unit integrally associated with Sarcospan in the dystrophin-glycoprotein complex and it is also linked to the signaling protein, neural nitric oxide synthase, through α -Syntrophin that associated with dystrobrevin.

REFERENCES

1. Heighway, J., et al. 1996. Coamplification in tumors of KRAS2, type 2 inositol 1, 4, 5 triphosphate receptor gene, and a novel human gene, KRAG. *Genomics* 35: 207-214.
2. Crosbie, R.H., et al. 1997. Sarcospan, the 25-kDa transmembrane component of the Dystrophin-glycoprotein complex. *J. Biol. Chem.* 272: 31221-31224.
3. Grady, R.M., et al. 2000. Maturation and maintenance of the neuromuscular synapse: genetic evidence for roles of the Dystrophin-glycoprotein complex. *Neuron* 25: 279-293.
4. Lebakken, C.S., et al. 2000. Sarcospan-deficient mice maintain normal muscle function. *Mol. Cell. Biol.* 20: 1669-1677.
5. Barresi, R., et al. 2000. Expression of γ -sarcoglycan in smooth muscle and its interaction with the smooth muscle sarcoglycan-Sarcospan complex. *J. Biol. Chem.* 275: 38554-38560.

CHROMOSOMAL LOCATION

Genetic locus: SSPN (human) mapping to 12p12.1.

PRODUCT

Sarcospan 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Sarcospan shRNA Plasmid (h): sc-43426-SH and Sarcospan shRNA (h) Lentiviral Particles: sc-43426-V as alternate gene silencing products.

For independent verification of Sarcospan (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-43426A, sc-43426B and sc-43426C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Sarcospan siRNA (h) is recommended for the inhibition of Sarcospan 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 μ M in 66 μ l. 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

Sarcospan (E-2): sc-393187 is recommended as a control antibody for monitoring of Sarcospan 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 κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Sarcospan gene expression knockdown using RT-PCR Primer: Sarcospan (h)-PR: sc-43426-PR (20 μ l). 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.