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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

SANTA CRUZ BIOTECHNOLOGY, INC.

Nebulette siRNA (m): sc-149894

BACKGROUND

Nebulin and nebulette are homologous thin-filament associated proteins expressed in skeletal and cardiac muscles, respectively. Nebulette, unlike nebulin, is confined to the Z-disc region of the sarcomere and does not span the whole thin filament length. Nebulette colocalizes with α-actinin in the pre-, nascent, and mature myofibrils. A polymorphism in the actin-binding motif of nebulette is a genetic marker of susceptibility to nonfamilial idiopathic dilated cardiomyopathy (IDC), characterized by a thin-walled heart and systolic dysfunction.

REFERENCES

1. Moncman, C.L., et al. 1995. Nebulette: a 107 kD nebulin-like protein in cardiac muscle. Cell Motil. Cytoskeleton 32: 205-225.
2. Millevoi, S., et al. 1998. Characterization of nebulette and nebulin and emerging concepts of their roles for vertebrate Z-discs. J. Mol. Biol. 282: 111-123.
3. Moncman, C.L., et al. 1999. Functional dissection of nebulette demonstrates actin binding of nebulin-like repeats and Z-line targeting of SH3 and linker domains. Cell Motil. Cytoskeleton 44: 1-22.
4. Arimura, T., et al. 2000. Characterization of the human nebulette gene: a polymorphism in an actin-binding motif is associated with nonfamilial idiopathic dilated cardiomyopathy. Hum. Genet. 107: 440-451.
5. Moncman, C.L., et al. 2000. Architecture of the thin filament-Z-line junction: lessons from nebulette and nebulin homologies. J. Muscle Res. Cell Motil. 21: 153-169.
6. Fock, U., et al. 2002. Nebulin is a thin filament protein of the cardiac muscle of the agnthans. J. Muscle Res. Cell Motil. 23: 205-213.
7. Ogut, O., et al. 2003. Interactions between nebulin-like motifs and thin filament regulatory proteins. J. Biol. Chem. 278: 3089-3097.

CHROMOSOMAL LOCATION

Genetic locus: Nebl (mouse) mapping to 2 A3.

PRODUCT

Nebulette siRNA (m) is a pool of 2 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 Nebulette shRNA Plasmid (m): sc-149894-SH and Nebulette shRNA (m) Lentiviral Particles: sc-149894-V as alternate gene silencing products.

For independent verification of Nebulette (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-149894A and sc-149894B.

PROTOCOLS

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

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

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

Nebulette (G-9): sc-393784 is recommended as a control antibody for monitoring of Nebulette 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 Nebulette gene expression knockdown using RT-PCR Primer: Nebulette (m)-PR: sc-149894-PR (20 µl). 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.