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SANTA CRUZ BIOTECHNOLOGY, INC.

N-RAP siRNA (m): sc-149773

BACKGROUND

Nebulin is a large filamentous protein specific to muscle tissue that may function as a "ruler" for Actin filament length. N-RAP (Nebulin-related anchoring protein) is a 1,730 amino acid protein that may play a role in myofibril assembly and in mediating force transmission in cardiomyocytes. N-RAP localizes to the intercalated disks of cardiac muscle and the myotendinous junctions of skeletal muscle, where it is exclusively expressed. Existing as four alternatively spliced isoforms, N-RAP contains one N-terminal LIM domain and fourty-four Nebulin repeats. N-RAP binds α-actinin and Filamin 2 during myofibril assembly and is also known to interact with Actin, KBTBD10, Talin-1 and vinculin. The gene encoding N-RAP maps to human chromosome 10q25.3 and mouse chromosome 19 D2.

REFERENCES

1. Luo, G., et al. 1997. Complete cDNA sequence and tissue localization of N-RAP, a novel Nebulin-related protein of striated muscle. Cell Motil. Cytoskeleton 38: 75-90.
2. Luo, G., et al. 1997. Mapping of the gene (NRAP) encoding N-RAP in the mouse and human genomes. Genomics 45: 229-232.
3. Online Mendelian Inheritance in Man, OMIM™. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 602873. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Luo, G., et al. 1999. Molecular interactions of N-RAP, a Nebulin-related protein of striated muscle myotendon junctions and intercalated disks. Biochemistry 38: 6135-6143.
5. Mohiddin, S.A., et al. 2003. Genomic organization, alternative splicing, and expression of human and mouse N-RAP, a Nebulin-related LIM protein of striated muscle. Cell Motil. Cytoskeleton 55: 200-212.
6. Lu, S., et al. 2003. New N-RAP-binding partners α-actinin, filamin and Krp1 detected by yeast two-hybrid screening: implications for myofibril assembly. J. Cell Sci. 116: 2169-2178.

CHROMOSOMAL LOCATION

Genetic locus: Nrap (mouse) mapping to 19 D2.

PRODUCT

N-RAP 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 N-RAP shRNA Plasmid (m): sc-149773-SH and N-RAP shRNA (m) Lentiviral Particles: sc-149773-V as alternate gene silencing products.

For independent verification of N-RAP (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-149773A, sc-149773B and sc-149773C.

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

N-RAP siRNA (m) is recommended for the inhibition of N-RAP 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

N-RAP (H-5): sc-514480 is recommended as a control antibody for monitoring of N-RAP 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 N-RAP gene expression knockdown using RT-PCR Primer: N-RAP (m)-PR: sc-149773-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.