SANTA CRUZ BIOTECHNOLOGY, INC.

Rootletin siRNA (m): sc-62961

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The Power to Question

BACKGROUND

Rootletin, also known as CROCC (ciliary rootlet coiled-coil protein), is a 2,017 amino acid protein that forms centriole-associated fibrous structures and is an essential component of the ciliary rootlet. Localized to basal bodies and centrosomes in ciliated and nonciliated cells, respectively, Rootletin associates with the proximal ends of basal bodies and, in photoreceptors, functions to form elongated polymers between them. Rootletin is required for centrosome cohesion and, through interaction with C-Nap1 (a centrosomal protein present at the ends of the centrioles), can regulate the linkage of centrioles to basal bodies. Rootletin exists as a homopolymer whose association with centrosomes can be regulated via phosphorylation by Nek2 (NIMA-related kinase 2). Two isoforms exist due to alternative splicing events.

REFERENCES

1. Yang, J., et al. 2002. Rootletin, a novel coiled-coil protein, is a structural component of the ciliary rootlet. J. Cell Biol. 159: 431-440.
2. Yang, J., et al. 2005. The ciliary rootlet maintains long-term stability of sensory cilia. Mol. Cell. Biol. 25: 4129-4137.
3. Bahe, S., et al. 2005. Rootletin forms centriole-associated filaments and functions in centrosome cohesion. J. Cell Biol. 171: 27-33.
4. Yang, J. and Li, T. 2006. Focus on molecules: Rootletin. Exp. Eye Res. 83: 1-2.
5. Yang, J., et al. 2006. Rootletin interacts with C-Nap1 and may function as a physical linker between the pair of centrioles/basal bodies in cells. Mol. Biol. Cell 17: 1033-1040.
6. Mi, J., et al. 2007. Protein phosphatase-1α regulates centrosome splitting through Nek2. Cancer Res. 67: 1082-1089.
7. Graser, S., et al. 2007. Cep68 and Cep215 (Cdk5rap2) are required for centrosome cohesion. J. Cell Sci. 120: 4321-4331.
8. Bahmanyar, S., et al. 2008. β-catenin is a Nek2 substrate involved in centrosome separation. Genes Dev. 22: 91-105.

CHROMOSOMAL LOCATION

Genetic locus: Crocc (mouse) mapping to 4 D3.

PRODUCT

Rootletin 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 Rootletin shRNA Plasmid (m): sc-62961-SH and Rootletin shRNA (m) Lentiviral Particles: sc-62961-V as alternate gene silencing products.

For independent verification of Rootletin (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-62961A, sc-62961B and sc-62961C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

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

Rootletin (C-2): sc-374056 is recommended as a control antibody for monitoring of Rootletin 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 Rootletin gene expression knockdown using RT-PCR Primer: Rootletin (m)-PR: sc-62961-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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