SANTA CRUZ BIOTECHNOLOGY, INC.

CARMIL siRNA (h): sc-62080

BACKGROUND

CARMIL, also referred to as Leucine-rich repeat containing 16 (LRRC16), is a member of a recently described family of leucine-rich repeat containing proteins which have a variety of functions throughout the body. CARMIL interacts with the Arp2/3 complex, the Actin capping protein CP and Myosin I to help assemble a multi-protein structure that is crucial to proper cell development. Through its interactions with these three proteins, CARMIL regulates capping of Actin filaments at the barbed end, nucleation of Actin by the Arp2/3 complex and Actin filament assembly by Myosin I, a barbed-end directed motor. Together, this complex generates the force for diverse cellular movements such as cytokinesis, phag-ocytosis and muscle contraction. Defects in the gene encoding CARMIL are thought to have various detrimental effects including reduced chemotactic aggregation, lowered rates of pinocytosis and inefficient assembly of the Myosin-Arp2/3-CP complex. Without proper CARMIL function, cell development is retarded due to improper Actin filament assembly.

REFERENCES

1. Jung, G., et al. 2001. The Dictyostelium CARMIL protein links capping protein and the Arp2/3 complex to type I myosins through their SH3 domains. J. Cell Biol. 153: 1479-1497.
2. Remmert, K., et al. 2004. CARMIL is a bona fide capping protein interactant. J. Biol. Chem. 279: 3068-3077.
3. Yang, C., et al. 2005. Mammalian CARMIL inhibits Actin filament capping by capping protein. Dev. Cell 9: 209-221.
4. Huang, M., et al. 2005. Presence of a novel inhibitor of capping protein in neutrophil extract. Cell Motil. Cytoskeleton 62: 232-243.
5. Uruno, T., et al. 2006. CARMIL is a potent capping protein antagonist: identification of a conserved CARMIL domain that inhibits the activity of capping protein and uncaps capped Actin filaments. J. Biol. Chem. 281: 10635-10650.
6. Bruck, S., et al. 2006. Identification of a novel inhibitory Actin-capping protein binding motif in CD2-associated protein. J. Biol. Chem. 281: 19196-19203.

CHROMOSOMAL LOCATION

Genetic locus: LRRC16A (human) mapping to 6p22.2.

PRODUCT

CARMIL 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CARMIL shRNA Plasmid (h): sc-62080-SH and CARMIL shRNA (h) Lentiviral Particles: sc-62080-V as alternate gene silencing products.

For independent verification of CARMIL (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-62080A, sc-62080B and sc-62080C.

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

CARMIL siRNA (h) is recommended for the inhibition of CARMIL 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 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

CARMIL (E-10): sc-365314 is recommended as a control antibody for monitoring of CARMIL 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 CARMIL gene expression knockdown using RT-PCR Primer: CARMIL (h)-PR: sc-62080-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.