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SANTA CRUZ BIOTECHNOLOGY, INC.

M-RIP siRNA (m): sc-149201

BACKGROUND

M-RIP (myosin phosphatase Rho interacting protein), also known as MPRIP, p116Rip, RIP3 or RHOIP3, is a 1,025 amino acid cytoplasmic and cytoskeletal protein that is required for regulation of the actin cytoskeleton. M-RIP colocalizes with myosin binding subunit (MBS) to regulate the phosphorylation of myosin light chain, and colocalizes with F-Actin through its N-terminus in the cytoskeleton. M-RIP also interacts with and RhoA at actin stress fibers via its adjacent coiled coil domains. M-RIP is highly expressed in ovary, with moderate levels found in brain, heart, liver, lung, skeletal muscle, testis and kidney. M-RIP depletion causes an increase of stress fibers in smooth muscle cells, whereas M-RIP over-expression causes disassembly of stress fibers in neuronal cells. Containing two PH domains, M-RIP has multiple phosphorylated serine and threonine residues and exists as three isoforms which are produced by alternative splicing events.

REFERENCES

1. Gebbink, M.F., et al. 1997. Identification of a novel, putative Rho-specific GDP/GTP exchange factor and a Rho A-binding protein: control of neuronal morphology. J. Cell Biol. 137: 1603-1613.
2. Mulder, J., et al. 2003. p116Rip is a novel filamentous Actin-binding protein. J. Biol. Chem. 278: 27216-27223.
3. Surks, H.K., et al. 2003. Myosin phosphatase-Rho interacting protein. A new member of the Myosin phosphatase complex that directly binds Rho A. J. Biol. Chem. 278: 51484-51493.
4. Mulder, J., et al. 2004. p116Rip targets Myosin phosphatase to the Actin cytoskeleton and is essential for Rho A/ROCK-regulated neuritogenesis. Mol. Biol. Cell 15: 5516-5527.
5. Koga, Y. and Ikebe, M. 2005. p116Rip decreases myosin II phosphorylation by activating myosin light chain phosphatase and by inactivating RhoA. J. Biol. Chem. 280: 4983-4991.
6. Surks, H.K., et al. 2005. M-RIP targets Myosin phosphatase to stress fibers to regulate Myosin light chain phosphorylation in vascular smooth muscle cells. J. Biol. Chem. 280: 42543-42551.

CHROMOSOMAL LOCATION

Genetic locus: Mprip (mouse) mapping to 11 B1.3.

PRODUCT

M-RIP 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 M-RIP shRNA Plasmid (m): sc-149201-SH and M-RIP shRNA (m) Lentiviral Particles: sc-149201-V as alternate gene silencing products.

For independent verification of M-RIP (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-149201A, sc-149201B and sc-149201C.

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

M-RIP siRNA (m) is recommended for the inhibition of M-RIP 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

M-RIP (E-1): sc-515720 is recommended as a control antibody for monitoring of M-RIP 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 M-RIP gene expression knockdown using RT-PCR Primer: M-RIP (m)-PR: sc-149201-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.

PROTOCOLS

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