SANTA CRUZ BIOTECHNOLOGY, INC.

MRCKα siRNA (m): sc-60059

BACKGROUND

Protein kinases comprise a large group of encoded factors that regulate cellular processes by catalyzing the transfer of a phosphate group to a hydroxyl acceptor in serine, threonine or tyrosine residues. Myotonic dystrophy kinase-related Cdc42-binding (DMPK-like) kinases α and β (MRCKα, β) contain a cysteine-rich motif and a putative pleckstrin homology domain. MRCKs can phosphorylate nonmuscle myosin light chain and influences actin-myosin contractility. MRCKα can phosphorylate and activate LIM kinases downstream of Cdc42, which leads to inactivation of ADF/cofilin and to Actin cytoskeletal reorganization. MRCKα can also influence neurite outgrowth promoted by Cdc42 and Rac.

REFERENCES

1. Hunter, T. 1995. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. Cell 80: 225-236.
2. Leung, T., Chen, X.Q., Tan, I., Manser, E. and Lim, L. 1998. Myotonic dystrophy kinase-related Cdc42-binding kinase acts as a Cdc42 effector in promoting cytoskeletal reorganization. Mol. Cell. Biol. 18: 130-140.
3. Moncrieff, C.L., Bailey, M.E., Morrison, N. and Johnson, K.J. 1999. Cloning and chromosomal localization of human Cdc42-binding protein kinase β. Genomics 57: 297-300.
4. Chen, X.Q., Tan, I., Leung, T. and Lim, L. 1999. The myotonic dystrophy kinase-related Cdc42-binding kinase is involved in the regulation of neurite outgrowth in PC12 cells. J. Biol. Chem. 274: 19901-19905.
5. Hunter, T. 2000. Signaling—2000 and beyond. Cell 100: 113-127.
6. Sumi, T., Matsumoto, K., Shibuya, A. and Nakamura, T. 2001. Activation of LIM kinases by myotonic dystrophy kinase-related Cdc42-binding kinase α. J. Biol. Chem. 276: 23092-23096.

CHROMOSOMAL LOCATION

Genetic locus: Cdc42bpa (mouse) mapping to 1 H4.

PRODUCT

MRCKα 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 MRCKα shRNA Plasmid (m): sc-60059-SH and MRCKα shRNA (m) Lentiviral Particles: sc-60059-V as alternate gene silencing products.

For independent verification of MRCKα (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-60059A, sc-60059B and sc-60059C.

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.

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

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

MRCKα (B-3): sc-374568 is recommended as a control antibody for monitoring of MRCKα 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 MRCKα gene expression knockdown using RT-PCR Primer: MRCKα (m)-PR: sc-60059-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.