SANTA CRUZ BIOTECHNOLOGY, INC.

MRCKγ siRNA (h): sc-96278

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. MRCKγ (serine/threonine-protein kinase MRCKγ), also known as CDC42BPG (Cdc42 binding protein kinase γ (DMPK-like)), myotonic dystrophy protein kinase-like α, DMPK2, HSMDPKIN or KAPPA-200, is a 1,551 amino acid cytoplasmic protein belonging to the protein kinase superfamily. Expressed in skeletal muscle and heart, MRCKγ exists as both a homodimer and homotetramer. MRCKγ may function as a downstream effector of Cdc42 in cytoskeletal reorganization, and is known to regulate the phosphorylation of MYPT1 and MYL2, which is required for actomyosin contractility in cell invasion. MRCKγ binds magnesium as a cofactor and strongly associates with GTP-bound Cdc42.

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., et al. 1998. Myotonic dystrophy kinase-related Cdc42-binding kinase acts as a Cdc42 effector in promoting cytoskeletal reorganization. Mol. Cell. Biol. 18: 130-140.
3. Nakamura, N., et al. 2000. Phosphorylation of ERM proteins at filopodia induced by Cdc42. Genes Cells 5: 571-581.
4. Ng, Y., et al. 2004. Expression of the human myotonic dystrophy kinase-related Cdc42-binding kinase γ is regulated by promoter DNA methylation and Sp1 binding. J. Biol. Chem. 279: 34156-34164.
5. Garcia, P., et al. 2006. Molecular insights into the self-assembly mechanism of dystrophia myotonica kinase. FASEB J. 20: 1142-1151.
6. Choi, S.H., et al. 2008. Characterization of the interaction of phorbol esters with the C1 domain of MRCK (myotonic dystrophy kinase-related Cdc42 binding kinase) α/β. J. Biol. Chem. 283: 10543-10549.

CHROMOSOMAL LOCATION

Genetic locus: CDC42BPG (human) mapping to 11q13.1.

PRODUCT

MRCKγ 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 MRCKγ shRNA Plasmid (h): sc-96278-SH and MRCKγ shRNA (h) Lentiviral Particles: sc-96278-V as alternate gene silencing products.

For independent verification of MRCKγ (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-96278A, sc-96278B and sc-96278C.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

MRCKγ siRNA (h) is recommended for the inhibition of MRCKγ 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 60 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

MRCKγ (C-20): sc-324140 is recommended as a control antibody for monitoring of MRCKγ 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MRCKγ gene expression knockdown using RT-PCR Primer: MRCKγ (h)-PR: sc-96278-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.