SANTA CRUZ BIOTECHNOLOGY, INC.

MYLK2 siRNA (m): sc-72101

BACKGROUND

The Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases) are a structurally related subfamily of serine/threonine kinases that includes CaMKI, CaMKII, CaMKIV and myosin light chain kinases (MYLK, also designated MLCK). The MYLK kinases phosphorylate myosin regulatory light chains to catalyze myosin interaction with Actin filaments resulting in contractile activity. Non-muscle, smooth muscle and skeletal/cardiac muscle MYLK isoforms exist. The MYLK gene (also designated MYLK1) encodes both smooth muscle and non-muscle isoforms as well as telokin, a small C-terminal isoform expressed only in smooth muscle with the capacity to stabilize unphosphorylated myosin filaments. Multiple transcript variants are described for the MYLK gene. Smooth-muscle and non-muscle MYLK isoforms are expressed in a wide variety of adult and fetal tissues. The skeletal/cardiac muscle isoforms of MYLK are encoded by a separate gene, MYLK2 (also designated skMLCK). MYLK appears to be a target for PAKs (p21-activated kinases). PAK1 interaction with MYLK results in a decrease in MYLK activity and myosin light chain phosphorylation.

REFERENCES

1. Roush, C.L., et al. 1988. Isolation of the cDNA encoding rat skeletal muscle myosin light chain kinase. Sequence and tissue distribution. J. Biol. Chem. 263: 10510-10516.
2. Haribabu, B., et al. 1995. Human calcium-calmodulin dependent protein kinase I: cDNA cloning, domain structure and activation by phosphorylation at threonine-177 by calcium-calmodulin dependent protein kinase I kinase. EMBO J. 14: 3679-3686.
3. Potier, M.C., et al. 1995. The human myosin light chain kinase (MLCK) from hippocampus: cloning, sequencing, expression, and localization to 3qcen-q21. Genomics 29: 562-570.
4. Garcia, J.G., et al. 1997. Myosin light chain kinase in endothelium: molecular cloning and regulation. Am. J. Respir. Cell Mol. Biol. 16: 489-494.
5. Sanders, L.C., et al. 1999. Inhibition of myosin light chain kinase by p21-activated kinase. Science 283: 2083-2085.
6. Lazar, V. and Garcia, J.G. 1999. A single human myosin light chain kinase gene (MLCK; MYLK). Genomics 57: 256-267.

CHROMOSOMAL LOCATION

Genetic locus: Mylk2 (mouse) mapping to 2 H1.

PRODUCT

MYLK2 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 MYLK2 shRNA Plasmid (m): sc-72101-SH and MYLK2 shRNA (m) Lentiviral Particles: sc-72101-V as alternate gene silencing products.

For independent verification of MYLK2 (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-72101A, sc-72101B and sc-72101C.

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

MYLK2 siRNA (m) is recommended for the inhibition of MYLK2 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MYLK2 gene expression knockdown using RT-PCR Primer: MYLK2 (m)-PR: sc-72101-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.