

MYPT1 siRNA (r): sc-156101

BACKGROUND

Myosin phosphatase target subunit 1 (MYPT1), also called Myosin-binding subunit of Myosin phosphatase, is one of the subunits and an integral component of the Myosin phosphatase. Myosin phosphatase regulates the interaction of Actin and Myosin downstream of the guanosine triphosphatase Rho, which inhibits Myosin phosphatase through the action of Rho-kinase. MYPT1 promoter contains one Sp1 transcription factor binding site, suggesting that MYPT1 is a housekeeping gene. Myotonic dystrophy protein kinase phosphorylates MYPT1 at tyrosine 654 to regulate Myosin II phosphorylation. Inhibition of Myosin light chain phosphatase results in Ca^{2+} sensitization of smooth muscle contraction. This inhibition is modulated through phosphorylation of MYPT1 by a ZIP-like kinase, which associates with MYPT1 and phosphorylates the inhibitory site in smooth muscle. The phosphorylation of MYPT1 by protein kinase C results in altered dephosphorylation of Myosin by attenuating the binding of protein phosphatase 1 catalytic subunit (PP1c) and the phosphorylated Myosin light chain to MYPT1. PP1c interacts at least four binding sites on the amino-terminus of MYPT1. A novel isoform of MYPT1, MYPT2, also interacts with PP1c. MYPT1 is localized on stress fibers, and it is distributed close to the cell membrane and at cell-cell contacts to regulate Myosin phosphatase activity.

REFERENCES

- Kimura, K., et al. 1996. Regulation of Myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* 273: 245-248.
- Takahashi, N., et al. 1997. Localization of the gene coding for Myosin phosphatase target subunit 1 (MYPT1) to human chromosome 12q15-q21. *Genomics* 44:150-152.
- Fujioka, M., et al. 1998. A new isoform of human Myosin phosphatase targeting/regulatory subunit (MYPT2): cDNA cloning, tissue expression, and chromosomal mapping. *Genomics* 49: 59-68.

CHROMOSOMAL LOCATION

Genetic locus: Ppp1r12a (rat) mapping to 7q21.

PRODUCT

MYPT1 siRNA (r) 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 MYPT1 shRNA Plasmid (r): sc-156101-SH and MYPT1 shRNA (r) Lentiviral Particles: sc-156101-V as alternate gene silencing products.

For independent verification of MYPT1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156101A, sc-156101B and sc-156101C.

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

MYPT1 siRNA (r) is recommended for the inhibition of MYPT1 expression in rat 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

p-MYPT1 (A-6): sc-377542 is recommended as a control antibody for monitoring of MYPT1 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 MYPT1 gene expression knockdown using RT-PCR Primer: MYPT1 (r)-PR: sc-156101-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

RESEARCH USE

For research use only, not for use in diagnostic procedures.