SANTA CRUZ BIOTECHNOLOGY, INC.

MYPT3 siRNA (m): sc-61131

BACKGROUND

The members of the MYPT family, MYPT1, MYPT2 and MYPT3 are the myosin-binding subunits of myosin phosphatase and an integral component of the myosin protein phosphatase. Myosin phosphatase regulates the interaction of actin and myosin downstream of the guanosine triphosphatase Rho. MYPT1 is localized on stress fibers and is distributed close to the cell membrane and at cell-cell contacts to regulate myosin phosphatase activity. In addition to MYPT1, a novel isoform of MYPT1, MYPT2, also interacts with PPIc. MYPT3, also designated PP16A, inhibits protein phosphatase activity involving phosphorylase, myosin light chain and myosin substrates. It acts as a lipid anchor and binds PP1. MYPT3 localizes primarily to the cell membrane.

REFERENCES

1. Skinner, J.A., et al. 2001. Cloning and identification of MYPT3: a prenylatable myosin targetting subunit of protein phosphatase 1. Biochem. J. 356: 257-267.
2. Cao, W., et al. 2002. TIMAP, a novel CAAX box protein regulated by TGF-β1 and expressed in endothelial cells. Am. J. Physiol., Cell Physiol. 283: C327-C337.
3. Ito, M., et al. 2004. Myosin phosphatase: structure, regulation and function. Mol. Cell. Biochem. 259: 197-209.
4. Vereshchagina, N., et al. 2004. The essential role of PP1β in *Drosophila* is to regulate nonmuscle myosin. Mol. Biol. Cell 15: 4395-4405.
5. SWISS-PROT/TrEMBL (Q96I34). World Wide Web URL: http://www.expasy.ch/sprot/sprot-top.html

CHROMOSOMAL LOCATION

Genetic locus: Ppp1r16a (mouse) mapping to 15 D3.

PRODUCT

MYPT3 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 MYPT3 shRNA Plasmid (m): sc-61131-SH and MYPT3 shRNA (m) Lentiviral Particles: sc-61131-V as alternate gene silencing products.

For independent verification of MYPT3 (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-61131A, sc-61131B and sc-61131C.

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

MYPT3 siRNA (m) is recommended for the inhibition of MYPT3 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

MYPT3 (E-6): sc-376946 is recommended as a control antibody for monitoring of MYPT3 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 MYPT3 gene expression knockdown using RT-PCR Primer: MYPT3 (m)-PR: sc-61131-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.