

MKP-3 siRNA (h): sc-39000

BACKGROUND

Mitogen-activated protein (MAP) kinases are a large class of proteins involved in signal transduction pathways that are activated by a range of stimuli and mediate a number of physiological and pathological changes in the cell. Dual specificity phosphatases (DSPs) are a subclass of the protein tyrosine phosphatase (PTP) gene superfamily, which are selective for dephosphorylating critical phosphothreonine and phosphotyrosine residues within MAP kinases. DSP gene expression is induced by a host of growth factors and/or cellular stresses, thereby negatively regulating MAP kinase superfamily members, including MAPK/ERK, SAPK/JNK and p38. The members of the dual-specificity phosphatase protein family include MKP-1/CL100 (3CH134), VHR, PAC1, MKP-2, hVH-3 (B23), hVH-5, MKP-3, MKP-X and MKP-4. Human MKP-3 maps to chromosome 12q21.33 and encodes a 381 amino acid protein that specifically inactivates members of the ERK family and is expressed in a variety of tissues with the highest levels in heart and pancreas.

REFERENCES

1. Keyse, S.M. 1995. An emerging family of dual specificity MAP kinase phosphatases. *Biochim. Biophys. Acta* 1265: 152-160.
2. Muda, M., et al. 1997. Molecular cloning and functional characterization of a novel mitogen-activated protein kinase phosphatase, MKP-4. *J. Biol. Chem.* 272: 5141-5151.
3. Sun, H. 1998. Functional studies of dual-specificity phosphatases. *Methods Mol. Biol.* 84: 307-318.
4. Online Mendelian Inheritance in Man, OMIM™. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 603068. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: DUSP6 (human) mapping to 12q21.33.

PRODUCT

MKP-3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MKP-3 shRNA Plasmid (h): sc-39000-SH and MKP-3 shRNA (h) Lentiviral Particles: sc-39000-V as alternate gene silencing products.

For independent verification of MKP-3 (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-39000A, sc-39000B and sc-39000C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

MKP-3 siRNA (h) is recommended for the inhibition of MKP-3 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 μ M in 66 μ l. 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

MKP-3 (F-12): sc-377070 is recommended as a control antibody for monitoring of MKP-3 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 MKP-3 gene expression knockdown using RT-PCR Primer: MKP-3 (h)-PR: sc-39000-PR (20 μ l, 428 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lee, W.J., et al. 2014. N ϵ -carboxymethyllysine-mediated endoplasmic reticulum stress promotes endothelial cell injury through Nox4/MKP-3 interaction. *Free Radic. Biol. Med.* 74: 294-306.
2. Kawashima, I., et al. 2015. Negative regulation of the LKB1/AMPK pathway by ERK in human acute myeloid leukemia cells. *Exp. Hematol.* 43: 524-533.
3. Boulding, T., et al. 2016. Differential roles for DUSP family members in epithelial-to-mesenchymal transition and cancer stem cell regulation in breast cancer. *PLoS ONE* 11: e0148065.

RESEARCH USE

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