SANTA CRUZ BIOTECHNOLOGY, INC.

MKP-6 siRNA (h): sc-61050

BACKGROUND

The deduced 198 amino acid MAP kinase phosphatase 6 (MKP-6), also designated MAP6 and dual-specificity phosphatase 14 (DUSP14), is homologous to other MKP family proteins in that it has a conserved, centrally located, catalytic core, but differs from traditional MKP proteins because it contains unique N- and C-terminal regions. Binding and deletion analyses have established that the interaction between the cytoplasmic tail of CD28 (a T cell antigen) and MKP-6 occurs at Tyr 200 of CD28 and is specific for both MKP-6 and CD28; however, Tyr 200 can be mutated to Phe 200 without a loss of binding ability. Functional analysis indicates that MKP-6 dephosphorylates ERK, JNK and p38 while acting as a negative regulator of CD28 signaling. MKP-6 is expressed ubiquitously, although expression is stronger in certain cell types and tissues than in others.

REFERENCES

1. Tanoue, T., et al. 2001. A Novel MAPK phosphatase MKP-7 acts preferentially on JNK/SAPK and p38 α and β MAPKs. J. Biol. Chem. 276: 26629-26639.
2. Marti, F., et al. 2001. Negative-feedback regulation of CD28 costimulation by a novel mitogen-activated protein kinase phosphatase, MKP-6. J. Immunol. 166: 197-206.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606618. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Nyati, M.K., et al. 2006. Ataxia telangiectasia mutated down-regulates phospho-extracellular signal-regulated kinase 1/2 via activation of MKP-1 in response to radiation. Cancer Res. 66: 11554-11559.
5. Nakano, Y. 2007. Novel function of DUSP14/MKP-6 (dual specific phosphatase 14) as a nonspecific regulatory molecule for delayed-type hypersensitivity. Br. J. Dermatol. 156: 848-860.
6. Klinger, S., et al. 2008. Increasing GLP-1-induced b cell proliferation by silencing the negative regulators of signaling cAMP response element modulator-α and DUSP14. Diabetes 57: 584-593.
7. Elass, E., et al. 2008. Mycobacterial lipomannan induces MAP kinase phosphatase-1 expression in macrophages. FEBS Lett. 582: 445-450.

CHROMOSOMAL LOCATION

Genetic locus: DUSP14 (human) mapping to 17q12.

PRODUCT

MKP-6 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 MKP-6 shRNA Plasmid (h): sc-61050-SH and MKP-6 shRNA (h) Lentiviral Particles: sc-61050-V as alternate gene silencing products.

For independent verification of MKP-6 (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-61050A, sc-61050B and sc-61050C.

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

MKP-6 siRNA (h) is recommended for the inhibition of MKP-6 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 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

MKP-6 (4B5-E6): sc-517023 is recommended as a control antibody for monitoring of MKP-6 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 MKP-6 gene expression knockdown using RT-PCR Primer: MKP-6 (h)-PR: sc-61050-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.