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MKP-1 siRNA (h2): sc-44312

BACKGROUND

A key element in the pathway involved in the transduction of signals from activated protein-tyrosine kinase transmembrane receptors has been identified as the family of mitogen-activated protein kinases (MAP kinases). The most well known of these Ser/Thr kinases are ERK 1 and ERK 2. Mitogenic stimulation of cells triggers the activation of MAP kinases through phosphorylation of both tyrosyl (Y185) and threonyl (T183) residues. Phosphorylation of the T183 and Y185 ERK regulatory site is mediated by MAP kinase (MEK), which in turn is regulated by the proto-oncogene product Raf. Two highly related phosphatases, designated MKP-1 and MKP-2, exhibit 59% sequence identity at the amino acid level and oppose the action of MEK by downregulating the kinase activity of ERK 1 and ERK 2. MAP kinase phosphatase-1 and -2 proteins function by dephosphorylating ERK 1 and ERK 2 at their T-E-Y regulatory motif. An additional phosphatase encoded by the DUSP2 gene, designated PAC-1, also functions to downregulate ERK 1 and ERK 2 kinase activity. PAC-1 is a nuclear protein whose expression is strongly induced in response to mitogen.

REFERENCES

1. Cobb, M.H., et al. 1991. Extracellular signal-regulated kinases: ERKs in progress. *Cell Reg.* 2: 965-978.
2. Payne, D.M., et al. 1991. Identification of the regulatory phosphorylation sites in p42/mitogen-activated protein kinase (MAP) kinase. *EMBO J.* 10: 885-892.
3. Ahn, N.G., et al. 1992. The mitogen-activated protein kinase activator. *Curr. Opin. Cell Biol.* 4: 992-999.
4. Nishida, E., et al. 1993. The MAP kinase cascade is essential for diverse signal transduction pathways. *Trends Biochem. Sci.* 18: 128-131.
5. Sun, H., et al. 1993. MKP-1 (3CH134), an immediate early gene product is a dual specificity phosphatase that dephosphorylates MAP kinase *in vivo*. *Cell* 75: 487-493.

CHROMOSOMAL LOCATION

Genetic locus: DUSP1 (human) mapping to 5q35.1.

PRODUCT

MKP-1 siRNA (h2) 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-1 shRNA Plasmid (h2): sc-44312-SH and MKP-1 shRNA (h2) Lentiviral Particles: sc-44312-V as alternate gene silencing products.

For independent verification of MKP-1 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44312A, sc-44312B and sc-44312C.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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 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-1 siRNA (h2) is recommended for the inhibition of MKP-1 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-1 (E-6): sc-373841 is recommended as a control antibody for monitoring of MKP-1 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MKP-1 gene expression knockdown using RT-PCR Primer: MKP-1 (h2)-PR: sc-44312-PR (20 μ l, 449 bp). 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.