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# MKP-1 shRNA (h2) Lentiviral Particles: sc-44312-V

## 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 shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see MKP-1 siRNA (h2): sc-44312 and MKP-1 shRNA Plasmid (h2): sc-44312-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ\text{C}$ . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ\text{C}$  for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

MKP-1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of MKP-1 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## 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  $\mu$ l, 449 bp). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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