

# MEK-4 siRNA (h): sc-35909

## BACKGROUND

A family of protein kinases located upstream of the MAP kinases and responsible for their activation has been identified. The prototype member of this family, designated MAP kinase kinase, or MEK-1, specifically phosphorylates the MAP kinase regulatory threonine and tyrosine residues present in the Thr-Glu-Tyr motif of ERK. A second MEK family member, MEK-2, resembles MEK-1 in its substrate specificity. MEK-3 (or MKK-3) functions to activate p38 MAP kinase, and MEK-4 (also called SEK1 or MKK-4) activates both p38 and JNK MAP kinases. MEK-5 appears to specifically phosphorylate ERK 5, whereas MEK-6 phosphorylates p38 and p38 $\beta$ . MEK-7 (or MKK-7) phosphorylates and activates the JNK signal transduction pathway.

## REFERENCES

1. Crews, C.M., et al. 1992. The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product. *Science* 258: 478-480.
2. Wu, J., et al. 1993. Identification and characterization of a new mammalian mitogen-activated protein kinase kinase, MKK2. *Mol. Cell. Biol.* 13: 4539-4548.
3. Derijard, B., et al. 1995. Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. *Science* 267: 682-685.
4. Zhou, G., et al. 1995. Components of a new human protein kinase signal transduction pathway. *J. Biol. Chem.* 270: 12665-12669.

## CHROMOSOMAL LOCATION

Genetic locus: MAP2K4 (human) mapping to 17p12.

## PRODUCT

MEK-4 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MEK-4 shRNA Plasmid (h): sc-35909-SH and MEK-4 shRNA (h) Lentiviral Particles: sc-35909-V as alternate gene silencing products.

For independent verification of MEK-4 (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-35909A, sc-35909B and sc-35909C.

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

## APPLICATIONS

MEK-4 siRNA (h) is recommended for the inhibition of MEK-4 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  $\mu$ M in 66  $\mu$ 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

MEK-4 (G-7): sc-376838 is recommended as a control antibody for monitoring of MEK-4 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MEK-4 gene expression knockdown using RT-PCR Primer: MEK-4 (h)-PR: sc-35909-PR (20  $\mu$ l, 422 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Hou, C.H., et al. 2008. The novel isoflavone 7-hydroxy-3',4'-benzoisoflavone induces cell apoptosis in human osteosarcoma cells. *Cancer Lett.* 271: 117-128.
2. Marasa, B.S., et al. 2009. Increased MKK4 abundance with replicative senescence is linked to the joint reduction of multiple microRNAs. *Sci. Signal.* 2: ra69.
3. Jarvis, I.W., et al. 2014. Nanomolar levels of PAHs in extracts from urban air induce MAPK signaling in Hep G2 cells. *Toxicol. Lett.* 229: 25-32.
4. Hasna, J., et al. 2018. Orai3 calcium channel and resistance to chemotherapy in breast cancer cells: the p53 connection. *Cell Death Differ.* 25: 691-705.
5. Xu, Q., et al. 2022. *Fusobacterium nucleatum* induces excess methyltransferase-like 3-mediated microRNA-4717-3p maturation to promote colorectal cancer cell proliferation. *Cancer Sci.* E-published.

## RESEARCH USE

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