

MEK kinase-4 siRNA (m): sc-35903

BACKGROUND

Mitogen-activated protein (MAP) kinase cascades are activated by various extracellular stimuli including growth factors. The MEK kinases (also called MAP kinase kinase kinases) phosphorylate and activate the MAP kinases, including ERK, JNK and p38. The MEK kinases characterized to date include Raf-1, Raf-B, MOS, MEK kinase-1, MEK kinase-2, MEK kinase-3, MEK kinase-4 and ASK 1 (also designated MEK kinase-5). MEK kinase-1 has been shown to phosphorylate MEK-1 via a Raf-independent pathway. Evidence suggests that MEK-3 is preferentially activated by MEK kinase-3 and that MEK-4 is activated by both MEK kinase-2 and MEK kinase-3. MEK kinase-4 has been shown to specifically activate the JNK pathway. ASK1 activates both MEK-4 and MEK-3/MEK-6 pathways.

REFERENCES

1. Lange-Carter, C.A., et al. 1993. A divergence in the MAP kinase regulatory network defined by MEK kinase and Raf. *Science* 260: 315-319.
2. Guan, K.L. 1994. The mitogen activated protein kinase signal transduction pathway: from the cell surface to the nucleus. *Cell. Signal.* 6: 581-589.
3. Wang, X.S., et al. 1996. Molecular cloning and characterization of a novel protein kinase with a catalytic domain homologous to mitogen-activated protein kinase kinase kinase. *J. Biol. Chem.* 271: 31607-31611.
4. Fanger, G.R., et al. 1997. MEK kinases are regulated by EGF and selectively interact with Rac/Cdc42. *EMBO J.* 16: 4961-4972.
5. Gerwins, P., et al. 1997. Cloning of a novel mitogen-activated protein kinase kinase kinase, MEKK4, that selectively regulates the c-Jun amino terminal kinase pathway. *J. Biol. Chem.* 272: 8288-8295.

CHROMOSOMAL LOCATION

Genetic locus: Map3k4 (mouse) mapping to 17 A1.

PRODUCT

MEK kinase-4 siRNA (m) 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 MEK kinase-4 shRNA Plasmid (m): sc-35903-SH and MEK kinase-4 shRNA (m) Lentiviral Particles: sc-35903-V as alternate gene silencing products.

For independent verification of MEK kinase-4 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35903A, sc-35903B and sc-35903C.

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

MEK kinase-4 siRNA (m) is recommended for the inhibition of MEK kinase-4 expression in mouse 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

MEK kinase-4 (E-6): sc-166197 is recommended as a control antibody for monitoring of MEK kinase-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 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 MEK kinase-4 gene expression knockdown using RT-PCR Primer: MEK kinase-4 (m)-PR: sc-35903-PR (20 μ l, 471 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Bikkavilli, R.K., et al. 2008. G α_o mediates Wnt-JNK signaling through dishevelled 1 and 3, Rho A family members, and MEKK 1 and 4 in mammalian cells. *J. Cell Sci.* 121: 234-245.

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.