

MEK-5 siRNA (m): sc-35912

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 ERK5, whereas MEK-6 phosphorylates p38 and p38 β . 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.
5. Han, J., et al. 1996. Characterization of the structure and function of a novel MAP kinase kinase (Mkk6). *J. Biol. Chem.* 271: 2886-2891.
6. Jiang, Y., et al. 1996. Characterization of the structure and function of a new mitogen-activated protein kinase (p38 β). *J. Biol. Chem.* 271: 17920-17926.

CHROMOSOMAL LOCATION

Genetic locus: Map2k5 (mouse) mapping to 9 C.

PRODUCT

MEK-5 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-5 shRNA Plasmid (m): sc-35912-SH and MEK-5 shRNA (m) Lentiviral Particles: sc-35912-V as alternate gene silencing products.

For independent verification of MEK-5 (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-35912A, sc-35912B and sc-35912C.

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-5 siRNA (m) is recommended for the inhibition of MEK-5 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-5 (E-3): sc-365198 is recommended as a control antibody for monitoring of MEK-5 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-5 gene expression knockdown using RT-PCR Primer: MEK-5 (m)-PR: sc-35912-PR (20 μ l, 593 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Jo, M., et al. 2019. Inhibition of MEK5 suppresses TDP-43 toxicity via the mTOR-independent activation of the autophagy-lysosome pathway. *Biochem. Biophys. Res. Commun.* 513: 925-932.

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.