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MEK-3/6 shRNA (h) Lentiviral Particles: sc-43924-V

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 p38b. MEK-7 (or MKK-7) phosphorylates and activates the JNK signal transduction pathway.

REFERENCES

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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.
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CHROMOSOMAL LOCATION

Genetic locus: MAP2K3 (human) mapping to 17q11.2, MAP2K6 (human) mapping to 17q24.3.

PRODUCT

MEK-3/6 shRNA (h) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^9 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 MEK-3/6 siRNA (h): sc-43924 and MEK-3/6 shRNA Plasmid (h): sc-43924-SH as alternate gene silencing products.

STORAGE

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

APPLICATIONS

MEK-3/6 shRNA (h) Lentiviral Particles is recommended for the inhibition of MEK-3 and MEK-6 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

MEK-3/6 (B-1): sc-136982 is recommended as a control antibody for monitoring of MEK-3/6 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 MEK-3 and MEK-6 gene expression knockdown using RT-PCR Primer: MEK-3/6 (h)-PR: sc-43924-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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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