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SZABO-SCANDIC Handels GmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic)



MLK2 siRNA (m): sc-149467

BACKGROUND

Mixed lineage kinases are a family of protein kinases sharing two leucine zipper-like motifs, which are known to mediate protein dimerization, and a kinase domain whose primary structure is similar to both the tyrosine-specific and the serine/threonine-specific kinase classes. Members of the mixed-lineage kinase (MLK) family include MLK1, MLK2, MLK3 and dual leucine zipper kinase, also designated DLK. MLKs are expressed in neuronal cells where they are likely to interact between Rac1/Cdc42, MKK4 and MKK7 in death signaling. The MLK1 gene is expressed in epithelial tumor cell lines of the colon, breast and esophagus. The MLK2 gene encodes a predicted 954 amino acid, Src homology 3 (SH3) domain-containing protein. The MLK3 gene encodes an 847 amino acid, SH3 domain- and proline rich region-containing protein. Apoptosis mechanisms rely on MLKs as an upstream intermediate of mitochondrial cytochrome c release and caspase activation.

REFERENCES

1. Dorow, D.S., et al. 1993. Identification of a new family of human epithelial protein kinases containing two leucine/isoleucine-zipper domains. *Eur. J. Biochem.* 213: 701-710.
2. Ing, Y.L., et al. 1994. MLK-3: identification of a widely-expressed protein kinase bearing an SH3 domain and a leucine zipper-basic region domain. *Oncogene* 9: 1745-1750.
3. Dorow, D.S., et al. 1995. Complete nucleotide sequence, expression, and chromosomal localisation of human mixed-lineage kinase 2. *Eur. J. Biochem.* 234: 492-500.
4. Hirai, S., et al. 1997. MST/MLK2, a member of the mixed lineage kinase family, directly phosphorylates and activates SEK1, an activator of c-Jun N-terminal kinase/stress-activated protein kinase. *J. Biol. Chem.* 272: 15167-15173.
5. Leung, I.W., et al. 1998. Dimerization via tandem leucine zippers is essential for the activation of the mitogen-activated protein kinase kinase, MLK-3. *J. Biol. Chem.* 273: 32408-32415.

CHROMOSOMAL LOCATION

Genetic locus: Map3k10 (mouse) mapping to 7 A3.

PRODUCT

MLK2 siRNA (m) is a pool of 2 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 MLK2 shRNA Plasmid (m): sc-149467-SH and MLK2 shRNA (m) Lentiviral Particles: sc-149467-V as alternate gene silencing products.

For independent verification of MLK2 (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-149467A and sc-149467B.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

MLK2 siRNA (m) is recommended for the inhibition of MLK2 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

MLK2 (H-4): sc-393675 is recommended as a control antibody for monitoring of MLK2 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 MLK2 gene expression knockdown using RT-PCR Primer: MLK2 (m)-PR: sc-149467-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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