SANTA CRUZ BIOTECHNOLOGY, INC.

MLTK siRNA (h): sc-62625

BACKGROUND

MLTK (mitogen-activated protein kinase kinase kinase MLT, leucine zipper- and sterile α motif-containing kinase, HCCS-4) is an 800 amino acid protein encoded by the human gene ZAK. MLTK belongs to the protein kinase superfamily, STE Ser/Thr protein kinase family, MAP kinase kinase kinase subfamily and contains one protein kinase domain and one SAM (sterile α motif) domain. MLTK is a cytoplasmic protein found as a homodimer. It will translocate to the nucleus upon ultraviolet B irradiation. It is a stress-activated component of a protein kinase signal transduction cascade and helps regulates the JNK and p38 pathways. MLTK also has a role in the regulation of S and G_2 cell cycle checkpoint by direct phosphorylation of Chk2. Isoform 1 causes cell shrinkage and disruption of Actin stress fibers and may have a role in neoplastic cell transformation and cancer development. Isoform 1 also phosphorylates Histone H3 at Ser 28.

REFERENCES

1. Liu, T.C., et al. 2000. Cloning and expression of ZAK, a mixed lineage kinase-like protein containing a leucine-zipper and a sterile α motif. Biochem. Biophys. Res. Commun. 274: 811-816.
2. Gotoh, I., et al. 2001. Identification and characterization of a novel MAP kinase kinase kinase, MLTK. J. Biol. Chem. 276: 4276-4286.
3. Yang, J.J. 2002. Mixed lineage kinase ZAK utilizing MKK7 and not MKK4 to activate the c-Jun N-terminal kinase and playing a role in the cell arrest. Biochem. Biophys. Res. Commun. 297: 105-110.
4. Yang, J.J. 2003. A novel zinc finger protein, ZZaPK, interacts with ZAK and stimulates the ZAK-expressing cells re-entering the cell cycle. Biochem. Biophys. Res. Commun. 301: 71-77.
5. Takahashi, M., et al. 2003. Regulation of a mitogen-activated protein kinase kinase kinase, MLTK by PKN. J. Biochem. 133: 181-187.
6. Cho, Y.Y., et al. 2004. A novel role for mixed-lineage kinase-like mitogen-activated protein triple kinase α in neoplastic cell transformation and tumor development. Cancer Res. 64: 3855-3864.

CHROMOSOMAL LOCATION

Genetic locus: MAP3K20 (human) mapping to 2q31.1.

PRODUCT

MLTK 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MLTK shRNA Plasmid (h): sc-62625-SH and MLTK shRNA (h) Lentiviral Particles: sc-62625-V as alternate gene silencing products.

For independent verification of MLTK (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-62625A, sc-62625B and sc-62625C.

RESEARCH USE

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

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

MLTK siRNA (h) is recommended for the inhibition of MLTK 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 µM in 66 µl. 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

MLTK (A-2): sc-390924 is recommended as a control antibody for monitoring of MLTK 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 MLTK gene expression knockdown using RT-PCR Primer: MLTK (h)-PR: sc-62625-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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