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TLK1 siRNA (m): sc-45974

BACKGROUND

The Tousled-like kinases (TLK1 and TLK2, also designated PKU- β and PKU- α , respectively) are the human homologs of the Tousled gene from *Arabidopsis thaliana*, which encodes a serine/threonine kinase that is necessary for proper organ morphogenesis. Both TLKs contain a nuclear localization signal and a predicted coiled-coil region in the N terminal domain. TLK is ubiquitously expressed and is prevalent in mouse testis, especially in pachytene spermatocytes and round spermatids. TLK displays a propensity to dimerize through an interaction between its coiled-coil structure and is able to auto-phosphorylate, as well as phosphorylate exogenous substrates. TLK1 and TLK2 are regulated by the cell cycle, showing maximum activity during S phase. Subsequently, they are thought to regulate the development of multicellular organisms, including playing a key role in spermatogenesis, through a series of phosphorylations.

REFERENCES

1. Roe, J.L., Rivin, C.J., Sessions, R.A., Feldmann, K.A. and Zambryski, P.C. 1993. The Tousled gene in *Arabidopsis thaliana* encodes a protein kinase homolog that is required for leaf and flower development. *Cell* 75: 939-950.
2. Yamakawa, A., Kameoka, Y., Hashimoto, K., Yoshitake, Y., Nishikawa, K., Tanihara, K. and Date, T. 1997. cDNA cloning and chromosomal mapping of genes encoding novel protein kinases termed PKU- α and PKU- β , which have nuclear localization signal. *Gene* 202: 193-201.
3. Roe, J.L., Durfee, T., Zupan, J.R., Repetti, P.P., McLean, B.G. and Zambryski, P.C. 1997. Tousled is a nuclear serine/threonine protein kinase that requires a coiled-coil region for oligomerization and catalytic activity. *J. Biol. Chem.* 171: 5838-5845.
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5. Silje, H.H., Takahashi, K., Tanaka, K., Van Houwe, G. and Nigg, E.A. 1999. Mammalian homologues of the plant Tousled gene code for cell-cycle-regulated kinases with maximal activities linked to ongoing DNA replication. *EMBO J.* 18: 5691-5702.

CHROMOSOMAL LOCATION

Genetic locus: Tlk1 (mouse) mapping to 2 C2.

PRODUCT

TLK1 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 TLK1 shRNA Plasmid (m): sc-45974-SH and TLK1 shRNA (m) Lentiviral Particles: sc-45974-V as alternate gene silencing products.

For independent verification of TLK1 (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-45974A, sc-45974B and sc-45974C.

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

TLK1 siRNA (m) is recommended for the inhibition of TLK1 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

TLK1 (G-8): sc-393515 is recommended as a control antibody for monitoring of TLK1 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 TLK1 gene expression knockdown using RT-PCR Primer: TLK1 (m)-PR: sc-45974-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.