

TTK siRNA (m): sc-36759

BACKGROUND

Progression of cells through the cell cycle is regulated by variations in the levels and activities of a series of protein kinases as well as by oscillation in the levels of their regulatory subunits (i.e., cyclins). The full length sequence for a unique protein kinase of human origin, designated TTK, was cloned by screening a T cell expression library with anti-phosphotyrosine antibodies. Similarly, the mouse homolog of TTK was isolated from an embryonal carcinoma (EC) cell line by expression cloning. TTK/Esk are novel members of the Serine-Threonine/tyrosine family of protein kinases and are expressed in a broad range of proliferating human cells and tissues. TTK-Esk expression is reduced or absent in resting cells and in cells with a low proliferative index. When cells are induced to enter the cell cycle, levels of TTK mRNA, protein and kinase activity increase at the G₁ to S phase of the cell cycle and peak in the G₂ to M phase, suggesting that TTK/Esk may function as cell cycle regulatory components.

REFERENCES

1. Mills, G.B., et al. 1992. Expression of TTK, a novel human protein kinase, is associated with cell proliferation. *J. Biol. Chem.* 267: 16000-16006.
2. Douville, E.M., et al. 1992. Multiple cDNAs encoding the esk kinase predict transmembrane and intracellular enzyme isoforms. *Mol. Cell. Biol.* 12: 2681-2689.
3. Nurse, P. 1994. Ordering S phase and M phase in the cell cycle. *Cell* 79: 547-550.
4. Sherr, C.J. 1994. G₁ phase progression: cycling on cue. *Cell* 79: 551-555.
5. King, R.W., et al. 1994. Mitosis in transition. *Cell* 79: 563-571.
6. Hunter, T., et al. 1994. Cyclins and cancer II: cyclin D and CDK inhibitors come of age. *Cell* 79: 573-582.
7. Hogg, D., et al. 1994. Cell cycle dependent regulation of the protein kinase TTK. *Oncogene* 9: 89-96.

CHROMOSOMAL LOCATION

Genetic locus: Ttk (mouse) mapping to 9 E2.

PRODUCT

TTK 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 TTK shRNA Plasmid (m): sc-36759-SH and TTK shRNA (m) Lentiviral Particles: sc-36759-V as alternate gene silencing products.

For independent verification of TTK (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-36759A, sc-36759B and sc-36759C.

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

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

TTK (D-8): sc-376842 is recommended as a control antibody for monitoring of TTK 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 TTK gene expression knockdown using RT-PCR Primer: TTK (m)-PR: sc-36759-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.