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TTBK1 siRNA (m): sc-154747

BACKGROUND

TTBK1 (tau tubulin kinase 1), also known as BDK (brain-derived tau kinase), is a 1,321 amino acid protein that contains one protein kinase domain and belongs to the serine/threonine protein kinase family. Localized to the cytoplasm and expressed at high levels in brain and at lower levels in testis and spinal cord, TTBK1 functions as a serine/threonine kinase that can phosphorylate Tau (a protein involved in tubulin polymerization) on threonine, tyrosine and serine residues. Specifically, TTBK1 uses divalent cations, such as magnesium and manganese, to catalyze the ATP-dependent transfer of a phosphate group onto Tau, creating a phosphoprotein and ADP. Phosphorylation of Tau causes its aggregation and subsequent loss of function, suggesting an important role for TTBK1 in the control of tubulin dynamics. Two isoforms of TTBK1 are expressed due to alternative splicing events.

REFERENCES

1. Takahashi, M., et al. 1995. A novel tau-tubulin kinase from bovine brain. *FEBS Lett.* 372: 59-64.
2. Nagase, T., et al. 2001. Prediction of the coding sequences of unidentified human genes. XX. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. *DNA Res.* 8: 85-95.
3. Sato, S., et al. 2006. Tau-tubulin kinase 1 (TTBK1), a neuron-specific tau kinase candidate, is involved in tau phosphorylation and aggregation. *J. Neurochem.* 98: 1573-1584.
4. Kitano-Takahashi, M., et al. 2007. Expression, purification and crystallization of a human tau-tubulin kinase 2 that phosphorylates tau protein. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* 63: 602-604.
5. Nonnis, S., et al. 2008. Tau is endogenously nitrated in mouse brain: identification of a tyrosine residue modified *in vivo* by NO. *Neurochem. Res.* 33: 518-525.

CHROMOSOMAL LOCATION

Genetic locus: *Ttbk1* (mouse) mapping to 17 C.

PRODUCT

TTBK1 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 TTBK1 shRNA Plasmid (m): sc-154747-SH and TTBK1 shRNA (m) Lentiviral Particles: sc-154747-V as alternate gene silencing products.

For independent verification of TTBK1 (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-154747A, sc-154747B and sc-154747C.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

TTBK1 siRNA (m) is recommended for the inhibition of TTBK1 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

TTBK1 (B-6): sc-374600 is recommended as a control antibody for monitoring of TTBK1 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 TTBK1 gene expression knockdown using RT-PCR Primer: TTBK1 (m)-PR: sc-154747-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.