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TBCB siRNA (m): sc-154114

BACKGROUND

Microtubules, the primary component of the cytoskeletal network, are highly dynamic structures composed of α/β Tubulin heterodimers. Biosynthesis of functional microtubules involve the participation of several chaperones, termed Tubulin folding cofactors A (TBCA), B (TBCB), D (TBCD), E (TBCE) and C (TBCC), that act on folding intermediates downstream of the cytosolic chaperon, alternatively named TCP. TBCB (tubulin folding cofactor B), also known as CG22, CKAP1 or CKAPI, is a 244 amino acid cytoplasmic protein containing one CAP-Gly domain and is widely expressed. TBCB is involved in the regulation of tubulin heterodimer dissociation and may function as a negative regulator of axonal growth.

REFERENCES

1. Tian, G., et al. 1996. Pathway leading to correctly folded β -tubulin. *Cell* 86: 287-296.
2. Tian, G., et al. 1997. Tubulin subunits exist in an activated conformational state generated and maintained by protein cofactors. *J. Cell Biol.* 138: 821-832.
3. Grynberg, M., et al. 2003. Domain analysis of the tubulin cofactor system: a model for tubulin folding and dimerization. *BMC Bioinformatics* 4: 46.
4. Wang, W., et al. 2005. Gigaxonin interacts with tubulin folding cofactor B and controls its degradation through the ubiquitin-proteasome pathway. *Curr. Biol.* 15: 2050-2055.
5. Vadlamudi, R.K., et al. 2005. p21-activated kinase 1 regulates microtubule dynamics by phosphorylating tubulin cofactor B. *Mol. Cell. Biol.* 25: 3726-3736.
6. Kortazar, D., et al. 2007. Role of cofactors B (TBCB) and E (TBCE) in tubulin heterodimer dissociation. *Exp. Cell Res.* 313: 425-436.
7. Lopez-Fanarraga, M., et al. 2007. Tubulin cofactor B plays a role in the neuronal growth cone. *J. Neurochem.* 100: 1680-1687.
8. Fanarraga, M.L., et al. 2009. Tubulin cofactor B regulates microtubule densities during microglia transition to the reactive states. *Exp. Cell Res.* 315: 535-541.

CHROMOSOMAL LOCATION

Genetic locus: *Tbcb* (mouse) mapping to 7 B1.

PRODUCT

TBCB 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 TBCB shRNA Plasmid (m): sc-154114-SH and TBCB shRNA (m) Lentiviral Particles: sc-154114-V as alternate gene silencing products.

For independent verification of TBCB (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-154114A and sc-154114B.

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

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

TBCB (B-12): sc-377139 is recommended as a control antibody for monitoring of TBCB 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 TBCB gene expression knockdown using RT-PCR Primer: TBCB (m)-PR: sc-154114-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

RESEARCH USE

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