

β3 Tubulin siRNA (h): sc-105009

BACKGROUND

Tubulin is a major cytoskeleton component that has five distinct forms, designated α , β , γ , δ and ϵ Tubulin. α and β Tubulins form heterodimers which multimerize to form a microtubule filament. Multiple β Tubulin isoforms (β 1, β 2, β 3, β 4, β 5, β 6 and β 8) have been characterized and are expressed in mammalian tissues. β 1 and β 4 are present throughout the cytosol, β 2 is present in the nuclei and nucleoplasm, and β 3 is a neuron-specific cytoskeletal protein. γ Tubulin forms the gammasome, which is required for nucleating microtubule filaments at the centrosome. Both δ Tubulin and ϵ Tubulin are associated with the centrosome. δ Tubulin is a homolog of the *Chlamydomonas* δ Tubulin Uni3 and is found in association with the centrioles, whereas ϵ Tubulin localizes to the pericentriolar material. ϵ Tubulin exhibits a cell cycle-specific pattern of localization; first associating with only the older of the centrosomes in a newly duplicated pair, and later associating with both centrosomes.

REFERENCES

- Weisenberg, R. 1981. Invited review: the role of nucleotide triphosphate in Actin and Tubulin assembly and function. *Cell Motil.* 1: 485-497.
- Burns, R.G. 1991. α -, β -, and γ -Tubulins: sequence comparisons and structural constraints. *Cell Motil. Cytoskeleton* 20: 181-189.
- Zheng, Y., et al. 1991. γ Tubulin is present in *Drosophila melanogaster* and *Homo sapiens* and is associated with the centrosome. *Cell* 65: 817-823.
- Leask, A. and Stearns, T. 1998. Expression of amino- and carboxyl-terminal γ and β Tubulin mutants in cultured epithelial cells. *J. Biol. Chem.* 273: 2661-2668.

CHROMOSOMAL LOCATION

Genetic locus: TUBB3 (human) mapping to 16q24.3.

PRODUCT

β3 Tubulin siRNA (h) 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 β3 Tubulin shRNA Plasmid (h): sc-105009-SH and β3 Tubulin shRNA (h) Lentiviral Particles: sc-105009-V as alternate gene silencing products.

For independent verification of β3 Tubulin (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-105009A and sc-105009B.

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

β3 Tubulin siRNA (h) is recommended for the inhibition of β3 Tubulin 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 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

β3 Tubulin (2G10): sc-80005 is recommended as a control antibody for monitoring of β3 Tubulin 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 β3 Tubulin gene expression knockdown using RT-PCR Primer: β3 Tubulin (h)-PR: sc-105009-PR (20 μ l, 483 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Li, W., et al. 2014. Association of ABCB1, β Tubulin I, and III with multidrug resistance of MCF7/DOC subline from breast cancer cell line MCF7. *Tumour Biol.* 35: 8883-8891.
- Altonsy, M.O., et al. 2020. β3-Tubulin is critical for microtubule dynamics, cell cycle regulation, and spontaneous release of microvesicles in human malignant melanoma cells (A375). *Int. J. Mol. Sci.* 21: 1656.

RESEARCH USE

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

PROTOCOLS

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