δ Tubulin siRNA (m): sc-35158



The Power to Question

BACKGROUND

Tubulin is a major cytoskeleton component that has five distinct forms, designated $\alpha,\,\beta,\,\gamma,\,\delta$ and ϵ Tubulin. α and β Tubulins form heterodimers which multimerize to form a microtubule filament. Multiple β Tubulin isoforms ($\beta1,\,\beta2,\,\beta3,\,\beta4,\,\beta5,\,\beta6$ and $\beta8$) have been characterized and are expressed in mammalian tissues. $\beta1$ and $\beta4$ are present throughout the cytosol, $\beta2$ is present in the nuclei and nucleoplasm, and $\beta3$ 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 $\it{Chlamydomonas}\,\delta$ 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

- 1. 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.
- 3. Zheng, Y., et al. 1991. γ Tubulin is present in *Drosophila melangaster* and *Homo sapiens* and is associated with the centrosome. Cell 65: 817-823.
- 4. 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.
- Luduena, R.F. 1998. Multiple forms of Tubulin: different gene products and covalent modifications. Int. Rev. Cytol. 178: 207-275.
- Walss, C., et al. 1999. Presence of the β2 isotype of Tubulin in the nuclei of cultured mesangial cells from rat kidney. Cell Motil. Cytoskeleton 42: 274-284.
- 7. Modig, C., et al. 1999. Identification of β3 and β4 Tubulin isotypes in coldadapted microtubules from Atlantic cod (*Gadus morhua*): antibody mapping and cDNA sequencing. Cell Motil. Cytoskeleton 42: 315-330.

CHROMOSOMAL LOCATION

Genetic locus: Tubd1 (mouse) mapping to 11 C.

PRODUCT

 δ Tubulin 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see δ Tubulin shRNA Plasmid (m): sc-35158-SH and δ Tubulin shRNA (m) Lentiviral Particles: sc-35158-V as alternate gene silencing products.

For independent verification of δ Tubulin (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-35158A, sc-35158B and sc-35158C.

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

 δ Tubulin siRNA (m) is recommended for the inhibition of δ Tubulin 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 µM in 66 µl. 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

 δ Tubulin (A-1): sc-25259 is recommended as a control antibody for monitoring of δ 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-lgG κ BP-HRP: sc-516102 or m-lgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz MarkerTM Molecular Weight Standards: sc-2035, UltraCruz $^{\circ}$ Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-lgG κ BP-FITC: sc-516140 or m-lgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz $^{\circ}$ Mounting Medium: sc-24941 or UltraCruz $^{\circ}$ Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor δ Tubulin gene expression knockdown using RT-PCR Primer: δ Tubulin (m)-PR: sc-35158-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.

PROTOCOLS

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