

β5 Tubulin siRNA (m): sc-140335

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. There are five β Tubulin isoforms ($\beta 1$, $\beta 2$, $\beta 3$, $\beta 4A$ and $\beta 4B$) that are expressed in mammalian tissues. $\beta 1$ and $\beta 4$ are present throughout the cytosol, $\beta 2$ is present in the nuclei and nucleoplasm, and $\beta 3$ is a neuron-specific cytoskeletal protein. γ Tubulin forms the gammaosome, 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

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- Woulfe, J., et al. 2000. Tubulin immunoreactive neuronal intranuclear inclusions in the human brain. *Neuropathol. Appl. Neurobiol.* 26: 161-171.

CHROMOSOMAL LOCATION

Genetic locus: Tubb5 (mouse) mapping to 17 B1.

PRODUCT

$\beta 5$ 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see $\beta 5$ Tubulin shRNA Plasmid (m): sc-140335-SH and $\beta 5$ Tubulin shRNA (m) Lentiviral Particles: sc-140335-V as alternate gene silencing products.

For independent verification of $\beta 5$ 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-140335A, sc-140335B and sc-140335C.

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

$\beta 5$ Tubulin siRNA (m) is recommended for the inhibition of $\beta 5$ 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 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

$\beta 5$ Tubulin (708H2B): sc-517663 is recommended as a control antibody for monitoring of $\beta 5$ 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-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 $\beta 5$ Tubulin gene expression knockdown using RT-PCR Primer: $\beta 5$ Tubulin (m)-PR: sc-140335-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.