

# β5 Tubulin siRNA (h): sc-106649

## BACKGROUND

Tubulin is a major cytoskeleton component that has five distinct forms, designated  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$  Tubulin.  $\alpha$  and  $\beta$  Tubulins form heterodimers which multimerize to form a microtubule filament. Multiple  $\beta$  Tubulin isoforms ( $\beta 1$ ,  $\beta 2$ ,  $\beta 3$ ,  $\beta 4$ ,  $\beta 5$ ,  $\beta 6$  and  $\beta 8$ ) have been characterized and 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.  $\gamma$  Tubulin forms the gammasome, which is required for nucleating microtubule filaments at the centrosome. Both  $\delta$  Tubulin and  $\epsilon$  Tubulin are associated with the centrosome.  $\delta$  Tubulin is a homolog of the *Chlamydomonas*  $\delta$  Tubulin Uni3 and is found in association with the centrioles, whereas  $\epsilon$  Tubulin localizes to the pericentriolar material.  $\epsilon$  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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- Burns, R.G. 1991.  $\alpha$ ,  $\beta$ , and  $\gamma$  Tubulins: sequence comparisons and structural constraints. *Cell Motil. Cytoskeleton* 20: 181-189.
- Zheng, Y., Jung, M.K. and Oakley, B.R. 1991.  $\gamma$  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  $\gamma$  and  $\alpha$  Tubulin mutants in cultured epithelial cells. *J. Biol. Chem.* 273: 2661-2668.
- Ludueno, R.F. 1998. Multiple forms of Tubulin: different gene products and covalent modifications. *Int. Rev. Cytol.* 178: 207-275.
- Walss, C., Kreisberg, J.I. and Ludueno, R.F. 1999. Presence of the  $\beta$ -II isotype of Tubulin in the nuclei of cultured mesangial cells from rat kidney. *Cell Motil. Cytoskeleton* 42: 274-284.

## CHROMOSOMAL LOCATION

Genetic locus: TUBB (human) mapping to 6p21.33.

## PRODUCT

$\beta 5$  Tubulin siRNA (h) 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see  $\beta 5$  Tubulin shRNA Plasmid (h): sc-106649-SH and  $\beta 5$  Tubulin shRNA (h) Lentiviral Particles: sc-106649-V as alternate gene silencing products.

For independent verification of  $\beta 5$  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-106649A, sc-106649B and sc-106649C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

$\beta 5$  Tubulin siRNA (h) is recommended for the inhibition of  $\beta 5$  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  $\mu$ M in 66  $\mu$ 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> 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 (h)-PR: sc-106649-PR (20  $\mu$ 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](http://www.scbt.com) for detailed protocols and support products.