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# $\alpha$ Tubulin shRNA (h2) Lentiviral Particles: sc-44242-V

## 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

- 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.  $\alpha$ ,  $\beta$ , and  $\gamma$  Tubulins: sequence comparisons and structural constraints. *Cell Motil. Cytoskeleton* 20: 181-189.
- Zheng, Y., et al. 1991.  $\gamma$  Tubulin is present in *Drosophila melanogaster* and *Homo sapiens* and is associated with the centrosome. *Cell* 65: 817-823.
- Leask, A., et al. 1998. Expression of amino- and carboxyl-terminal  $\gamma$  and  $\beta$  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.

## CHROMOSOMAL LOCATION

Genetic locus: TUBA1B (human) mapping to 12q13.12.

## PRODUCT

$\alpha$  Tubulin shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see  $\alpha$  Tubulin siRNA (h2): sc-44242 and  $\alpha$  Tubulin shRNA Plasmid (h2): sc-44242-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

$\alpha$  Tubulin shRNA (h2) Lentiviral Particles is recommended for the inhibition of  $\alpha$  Tubulin expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

$\alpha$  Tubulin (A-21): sc-135659 is recommended as a control antibody for monitoring of  $\alpha$  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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor  $\alpha$  Tubulin gene expression knockdown using RT-PCR Primer:  $\alpha$  Tubulin (h2)-PR: sc-44242-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.