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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

α Tubulin siRNA (h2): sc-44242

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., et al. 1998. Expression of amino- and carboxyl-terminal γ and α Tubulin mutants in cultured epithelial cells. *J. Biol. Chem.* 273: 2661-2668.
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- Walss, C., et al. 1999. Presence of the β 2 isotype of Tubulin in the nuclei of cultured mesangial cells from rat kidney. *Cell Motil. Cytoskeleton* 42: 274-284.
- Modig, C., et al. 1999. Identification of β 3 and β 4 Tubulin isoforms in cold-adapted microtubules from Atlantic cod (*Gadus morhua*): antibody mapping and cDNA sequencing. *Cell Motil. Cytoskeleton* 42: 315-330.

PRODUCT

α Tubulin siRNA (h2) 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 α Tubulin shRNA Plasmid (h2): sc-44242-SH and α Tubulin shRNA (h2) Lentiviral Particles: sc-44242-V as alternate gene silencing products.

For independent verification of α Tubulin (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44242A, sc-44242B and sc-44242C.

PROTOCOLS

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

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

α Tubulin siRNA (h2) is recommended for the inhibition of α 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

α Tubulin (TU-02): sc-8035 is recommended as a control antibody for monitoring of α 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 α Tubulin gene expression knockdown using RT-PCR Primer: α Tubulin (h2)-PR: sc-44242-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Ishiguro, K., et al. 2011. Cutting edge: Tubulin α functions as an adaptor in NFAT-importin β interaction. *J. Immunol.* 186: 2710-2713.

RESEARCH USE

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