## SANTA CRUZ BIOTECHNOLOGY, INC.

# α Tubulin siRNA (h): sc-29188



## 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 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.

## PRODUCT

 $\alpha$  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  $\alpha$  Tubulin shRNA Plasmid (h): sc-29188-SH and  $\alpha$  Tubulin shRNA (h) Lentiviral Particles: sc-29188-V as alternate gene silencing products.

For independent verification of  $\alpha$  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-29188A, sc-29188B and sc-29188C.

## 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**

 $\alpha$  Tubulin siRNA (h) is recommended for the inhibition of  $\alpha$  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**

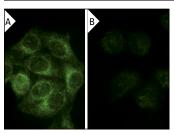
 $\alpha$  Tubulin (TU-02): sc-8035 is recommended as a control antibody for monitoring of  $\alpha$  Tubulin gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ 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  $\alpha$  Tubulin gene expression knockdown using RT-PCR Primer:  $\alpha$  Tubulin (h)-PR: sc-29188-PR (20  $\mu$ l, 530 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

### DATA



 $\alpha$  Tubulin siRNA (h): sc-29188. Immunofluorescence staining of methanol-fixed, control HeLa (A) and  $\alpha$  Tubulin siRNA silenced HeLa (B) cells showing diminished cytoskeletal staining in the siRNA silenced cells. Cells probed with  $\alpha$  Tubulin (H-300): sc-5546.

#### SELECT PRODUCT CITATIONS

- Joo, K.I., et al. 2008. Visualization of targeted transduction by engineered lentiviral vectors. Gene Ther. 15: 1384-1396.
- 2. Ishiguro, K., et al. 2011. Cutting edge: tubulin  $\alpha$  functions as an adaptor in NFAT-importin  $\beta$  interaction. J. Immunol. 186: 2710-2713.

## **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.