

# SPAG4 siRNA (h): sc-76544

## BACKGROUND

Mammalian sperm flagellum contain two cytoskeletal structures associated with the the axoneme: the outer dense fibers and the fibrous sheath. The outer dense fibers surround the axoneme in the midpiece and principal piece, whereas the fibrous sheath surrounds outer dense fibers of the tail of the principal piece. SPAG4 (sperm associated antigen 4), also known as outer dense fiber-associated protein SPAG4, is a 427 amino acid multi-pass membrane protein that may play a role in the organization and assembly of outer dense fibers (ODFs). Existing as a homodimer, SPAG4 interacts with Odf1 and is suggested to associate with microtubules. SPAG4 localizes to the transient manchette and axoneme of elongating spermatids and epididymal sperm. Containing one SUN domain, SPAG4 is considered a potential cancer marker.

## REFERENCES

1. Tarnasky, H., et al. 1998. A novel testis-specific gene, SPAG4, whose product interacts specifically with outer dense fiber protein ODF27, maps to human chromosome 20q11.2. *Cytogenet. Cell Genet.* 81: 65-67.
2. Shao, X., et al. 1999. SPAG4, a novel sperm protein, binds outer dense-fiber protein Odf1 and localizes to microtubules of manchette and axoneme. *Dev. Biol.* 211: 109-123.
3. Kierszenbaum, A.L. 2001. Spermatid manchette: plugging proteins to zero into the sperm tail. *Mol. Reprod. Dev.* 59: 347-349.
4. Shao, X., et al. 2001. Testicular protein SPAG5 has similarity to mitotic spindle protein Deepest and binds outer dense fiber protein Odf1. *Mol. Reprod. Dev.* 59: 410-416.
5. Zarsky, H.A., et al. 2003. Novel RING finger protein OIP1 binds to conserved amino acid repeats in sperm tail protein Odf1. *Biol. Reprod.* 68: 543-552.
6. Xing, X.W., et al. 2004. Identification of a novel gene SRG4 expressed at specific stages of mouse spermatogenesis. *Acta Biochim. Biophys. Sin.* 36: 351-359.
7. Kennedy, C., et al. 2004. Human sperm associated antigen 4 (SPAG4) is a potential cancer marker. *Cell Tissue Res.* 315: 279-283.

## CHROMOSOMAL LOCATION

Genetic locus: SPAG4 (human) mapping to 20q11.22.

## PRODUCT

SPAG4 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 SPAG4 shRNA Plasmid (h): sc-76544-SH and SPAG4 shRNA (h) Lentiviral Particles: sc-76544-V as alternate gene silencing products.

For independent verification of SPAG4 (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-76544A, sc-76544B and sc-76544C.

## RESEARCH USE

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

## 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

SPAG4 siRNA (h) is recommended for the inhibition of SPAG4 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

SPAG4 (H-6): sc-393115 is recommended as a control antibody for monitoring of SPAG4 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™ Molecular Weight Standards: sc-2035, UltraCruz® 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® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SPAG4 gene expression knockdown using RT-PCR Primer: SPAG4 (h)-PR: sc-76544-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## PROTOCOLS

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