

SPAG16 siRNA (m): sc-153703

BACKGROUND

Mammalian sperm flagellum contain two cytoskeletal structures associated with 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. SPAG16 (sperm associated antigen 16), also known as PF20 or WDR29, is a 631 amino acid cytoplasmic and cytoskeletal protein that interacts with the axoneme of sperm flagellum and the nucleus of postmeiotic germ cells. Required for sperm flagellar function, SPAG16 contains seven WD repeats and exists as five alternatively spliced isoforms. SPAG16 isoform 1 is expressed primarily in testis, while isoform 4 is found in testis, as well as brain, with lower levels in trachea, spinal cord, liver, thyroid, ovary, kidney, adrenal gland, pancreas and heart.

REFERENCES

1. Pennarun, G., Bridoux, A.M., Escudier, E., Dastot-Le Moal, F., Cacheux, V., Amselem, S. and Duriez, B. 2002. Isolation and expression of the human hPF20 gene orthologous to *Chlamydomonas* PF20: evaluation as a candidate for axonemal defects of respiratory cilia and sperm flagella. *Am. J. Respir. Cell Mol. Biol.* 26: 362-370.
2. Zhang, Z., Sapiro, R., Kapfhamer, D., Bucan, M., Bray, J., Chennathukuzhi, V., McNamara, P., Curtis, A., Zhang, M., Blanchette-Mackie, E.J. and Strauss, J.F. 2002. A sperm-associated WD repeat protein orthologous to *Chlamydomonas* PF20 associates with Spag6, the mammalian orthologue of *Chlamydomonas* PF16. *Mol. Cell. Biol.* 22: 7993-8004.
3. Zhang, Z., Kostetskii, I., Moss, S.B., Jones, B.H., Ho, C., Wang, H., Kishida, T., Gerton, G.L., Radice, G.L. and Strauss, J.F. 2004. Haploinsufficiency for the murine orthologue of *Chlamydomonas* PF20 disrupts spermatogenesis. *Proc. Natl. Acad. Sci. USA* 101: 12946-12951.
4. Zhang, Z., Zariwala, M.A., Mahadevan, M.M., Caballero-Campo, P., Shen, X., Escudier, E., Duriez, B., Bridoux, A.M., Leigh, M., Gerton, G.L., Kennedy, M., Amselem, S., Knowles, M.R. and Strauss, J.F. 2007. A heterozygous mutation disrupting the SPAG16 gene results in biochemical instability of central apparatus components of the human sperm axoneme. *Biol. Reprod.* 77: 864-871.
5. Wilson, C.W., Nguyen, C.T., Chen, M.H., Yang, J.H., Gacayan, R., Huang, J., Chen, J.N. and Chuang, P.T. 2009. Fused has evolved divergent roles in vertebrate Hedgehog signalling and motile ciliogenesis. *Nature* 459: 98-102.
6. Online Mendelian Inheritance in Man, OMIM[™]. 2009. Johns Hopkins University, Baltimore, MD. MIM Number: 612173. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: Spag16 (mouse) mapping to 1 C3.

PROTOCOLS

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

PRODUCT

SPAG16 siRNA (m) 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 SPAG16 shRNA Plasmid (m): sc-153703-SH and SPAG16 shRNA (m) Lentiviral Particles: sc-153703-V as alternate gene silencing products.

For independent verification of SPAG16 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-153703A, sc-153703B and sc-153703C.

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

SPAG16 siRNA (m) is recommended for the inhibition of SPAG16 expression in mouse 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SPAG16 gene expression knockdown using RT-PCR Primer: SPAG16 (m)-PR: sc-153703-PR (20 μ 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.