

SPAG5 siRNA (h): sc-76545

BACKGROUND

SPAG5 (sperm-associated antigen 5), also known as MAP126, hMAP126, DEEPEST or Astrin, is a 1,193 amino acid protein that is expressed at high levels in testis and at lower levels in liver, placenta, pancreas, colon and thymus. Localized specifically to spindle poles during prophase and throughout the spindle during metaphase and anaphase, SPAG5 is essential for proper spindle formation and is thought to be involved in the dynamic and functional regulation of mitotic spindles. Additionally, SPAG5 plays a role in sister chromatid cohesion and subsequent chromatid separation and, via its association with chromatids, is an important regulator of chromosome integrity. SPAG5 has a long stalk and a globular head domain through which it forms a homodimer that is characterized by aster-like structures. Upon DNA damage, SPAG5 may be phosphorylated by ATR or Atm.

REFERENCES

1. Chang, M.S., Huang, C.J., Chen, M.L., Chen, S.T., Fan, C.C., Chu, J.M., Lin, W.C. and Yang, Y.C. 2001. Cloning and characterization of hMAP126, a new member of mitotic spindle-associated proteins. *Biochem. Biophys. Res. Commun.* 287: 116-121.
2. Shao, X., Xue, J. and van der Hoorn, F.A. 2001. Testicular protein SPAG5 has similarity to mitotic spindle protein Deepest and binds outer dense fiber protein Odf1. *Mol. Reprod. Dev.* 59: 410-416.
3. Mack, G.J. and Compton, D.A. 2001. Analysis of mitotic microtubule-associated proteins using mass spectrometry identifies astrin, a spindle-associated protein. *Proc. Natl. Acad. Sci. USA* 98: 14434-14439.
4. Gruber, J., Harborth, J., Schnabel, J., Weber, K. and Hatzfeld, M. 2002. The mitotic spindle-associated protein astrin is essential for progression through mitosis. *J. Cell Sci.* 115: 4053-4059.
5. Yang, Y.C., Hsu, Y.T., Wu, C.C., Chen, H.T. and Chang, M.S. 2006. Silencing of astrin induces the p53-dependent apoptosis by suppression of HPV18 E6 expression and sensitizes cells to paclitaxel treatment in HeLa cells. *Biochem. Biophys. Res. Commun.* 343: 428-434.

CHROMOSOMAL LOCATION

Genetic locus: SPAG5 (human) mapping to 17q11.2.

PRODUCT

SPAG5 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SPAG5 shRNA Plasmid (h): sc-76545-SH and SPAG5 shRNA (h) Lentiviral Particles: sc-76545-V as alternate gene silencing products.

For independent verification of SPAG5 (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-76545A, sc-76545B and sc-76545C.

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

SPAG5 siRNA (h) is recommended for the inhibition of SPAG5 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

SPAG5 (LX-8): sc-100885 is recommended as a control antibody for monitoring of SPAG5 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 SPAG5 gene expression knockdown using RT-PCR Primer: SPAG5 (h)-PR: sc-76545-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.