SANTA CRUZ BIOTECHNOLOGY, INC.

SAS-6 siRNA (h): sc-76454

BACKGROUND

SAS-6 (spindle assembly abnormal protein 6 homolog, HsSAS-6) is a 657 amino acid protein encoded by the human gene SAS6. SAS-6 is a component of the centrosome that contains one PISA (present in SAS-6) domain. LK4, SAS-6, CPAP and other centriole related proteins are required at different stages of procentriole formation and were associated with different centriolar structures. SAS-6 associates only transiently with nascent procentrioles, whereas CEP135 and CPAP form a core structure within the proximal lumen of both parental and nascent centrioles. SAS-6 is necessary for procentriole formation in human cell lines and is localized asymmetrically next to the centriole at the onset of procentriole formation. SAS-6 levels oscillate during the cell cycle; it is degraded in mitosis starting at anaphase, and it accumulates again at the end of the following G_1 phase. The anaphase-promoting complex targets SAS-6 for degradation by the 26S Proteasome, and a KEN box in the C-terminus of SAS-6 is necessary for its degradation. Increased SAS-6 levels promoted the formation of multiple procentrioles forming next to a single centriole.

REFERENCES

1. Leidel, S., et al. 2005. SAS-6 defines a protein family required for centrosome duplication in *C. elegans* and in human cells. Nat. Cell Biol. 7: 115-125.
2. Leidel, S., et al. 2005. Centrosome duplication and nematodes: recent insights from an old relationship. Dev. Cell 9: 317-325.
3. Delattre, M., et al. 2006. Sequential protein recruitment in *C. elegans* centriole formation. Curr. Biol. 16: 1844-1849.
4. Pelletier, L., et al. 2006. Centriole assembly in *Caenorhabditis elegans*. Nature 444: 619-623.
5. Rodrigues-Martins, A., et al. 2007. DSAS-6 organizes a tube-like centriole precursor, and its absence suggests modularity in centriole assembly. Curr. Biol. 17: 1465-1472.
6. Peel, N., et al. 2007. Overexpressing centriole-replication proteins *in vivo* induces centriole overduplication and *de novo* formation. Curr. Biol. 17: 834-843.

CHROMOSOMAL LOCATION

Genetic locus: SASS6 (human) mapping to 1p21.2.

PRODUCT

SAS-6 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SAS-6 shRNA Plasmid (h): sc-76454-SH and SAS-6 shRNA (h) Lentiviral Particles: sc-76454-V as alternate gene silencing products.

For independent verification of SAS-6 (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-76454A, sc-76454B and sc-76454C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

SAS-6 siRNA (h) is recommended for the inhibition of SAS-6 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 µM in 66 µl. 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

SAS-6 (91.390.21): sc-81431 is recommended as a control antibody for monitoring of SAS-6 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® 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® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SAS-6 gene expression knockdown using RT-PCR Primer: SAS-6 (h)-PR: sc-76454-PR (20 µl). 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.

PROTOCOLS

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