SANTA CRUZ BIOTECHNOLOGY, INC.

SKAP siRNA (h): sc-90222

BACKGROUND

Interactions between kinetochore and spindle microtubules play a critical role in chromosome segregation during mitosis. SKAP (small kinetochore associated protein), also known as KNSTRN (kinetochore-localized astrin/SPAG5 binding protein), HSD11, C15orf23 or TRAF4AF1, is a 316 widely expressed nuclear and cytoplasmic protein that is an essential component of the mitotic spindle. Required for chromosome alignment during mitosis, SKAP regulates metaphase-to-anaphase transition, promotes normal timing of sister chromatid segregation and maintains spindle pole architecture. SKAP forms a complex with SPAG5 localizes to microtubule ends and stabilizes microtubule-kinetochore attachments. Mutations in the gene encoding SKAP may lead to cutaneous squamous cell carcinomas, a malignancy of the skin. SKAP is encoded by a gene located on human chromosome 15q15.1 and exists as three alternatively spliced isoforms. SKAP is down-regulated by nitric oxide.

REFERENCES

1. Turpaev, K., et al. 2005. Analysis of differentially expressed genes in nitric oxide-exposed human monocytic cells. Free Radic. Biol. Med. 38: 1392-1400.
2. Fang, L., et al. 2009. SKAP associates with kinetochores and promotes the metaphase-to-anaphase transition. Cell Cycle 8: 2819-2827.
3. Burkard, T.R., et al. 2011. Initial characterization of the human central proteome. BMC Syst. Biol. 5: 17.
4. Dunsch, A.K., et al. 2011. The astrin-kinastrin/SKAP complex localizes to microtubule plus ends and facilitates chromosome alignment. J. Cell Biol. 192: 959-968.
5. Huang, Y., et al. 2012. CENP-E kinesin interacts with SKAP protein to orchestrate accurate chromosome segregation in mitosis. J. Biol. Chem. 287: 1500-1509.
6. Wang, X., et al. 2012. Mitotic regulator SKAP forms a link between kinetochore core complex KMN and dynamic spindle microtubules. J. Biol. Chem. 287: 39380-39390.
7. Lee, C.S., et al. 2014. Recurrent point mutations in the kinetochore gene KNSTRN in cutaneous squamous cell carcinoma. Nat. Genet. 46: 1060-1062.

CHROMOSOMAL LOCATION

Genetic locus: KNSTRN (human) mapping to 15q15.1.

PRODUCT

SKAP 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 SKAP shRNA Plasmid (h): sc-90222-SH and SKAP shRNA (h) Lentiviral Particles: sc-90222-V as alternate gene silencing products.

For independent verification of SKAP (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-90222A, sc-90222B and sc-90222C.

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

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

SKAP (F-11): sc-514112 is recommended as a control antibody for monitoring of SKAP 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 SKAP gene expression knockdown using RT-PCR Primer: SKAP (h)-PR: sc-90222-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.