SANTA CRUZ BIOTECHNOLOGY, INC.

CENP-K siRNA (h): sc-91868

BACKGROUND

During mitosis, the transient assembly of the kinetochore occurs on a platform known as the centromere, a specialized chromatin structure that is comprised of various centromere proteins (CENPs). There are two multi-protein centromere complexes, known as CENPA-NAC (nucleosome-associated) and CENPA-CAD (nucleosome distal), which interact with one another to facilitate both the assembly and the activity of the centromere. CENP-K (centromere protein K), also known as P33, Solt, FKSG14, AF5α or ICEN37, is a 269 amino acid component of the CENPA-CAD complex. Localized exclusively to the centromere, CENP-K is thought to be involved in mitotic progression, chromosome segregation and the assembly and incorporation of kinetochore proteins into centromeres. CENP-K is expressed at high levels in fetal liver and at lower levels in adult placenta and lung. Defects in the gene encoding CENP-K are associated with acute leukemias, suggesting that CENP-K plays a role in carcinogenesis.

REFERENCES

1. Taki, T., et al. 1996. Fusion of the MLL gene with two different genes, AF-6 and AF-5α, by a complex translocation involving chromosomes 5, 6, 8 and 11 in infant leukemia. Oncogene 13: 2121-2130.
2. Yamashita, A., et al. 2000. Characterization of Solt, a novel SoxLZ/Sox6 binding protein expressed in adult mouse testis. FEBS Lett. 481: 147-151.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 611502. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Obuse, C., et al. 2004. Proteomics analysis of the centromere complex from HeLa interphase cells: UV-damaged DNA binding protein 1 (DDB-1) is a component of the CEN-complex, while BMI-1 is transiently co-localized with the centromeric region in interphase. Genes Cells 9: 105-120.
5. Izuta, H., et al. 2006. Comprehensive analysis of the ICEN (Interphase Centromere Complex) components enriched in the CENP-A chromatin of human cells. Genes Cells 11: 673-684.
6. Okada, M., et al. 2006. The CENP-H-I complex is required for the efficient incorporation of newly synthesized CENP-A into centromeres. Nat. Cell Biol. 8: 446-457.

CHROMOSOMAL LOCATION

Genetic locus: CENPK (human) mapping to 5q12.3.

PRODUCT

CENP-K 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 CENP-K shRNA Plasmid (h): sc-91868-SH and CENP-K shRNA (h) Lentiviral Particles: sc-91868-V as alternate gene silencing products.

For independent verification of CENP-K (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-91868A, sc-91868B and sc-91868C.

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

CENP-K siRNA (h) is recommended for the inhibition of CENP-K 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

CENP-K (40.3): sc-81831 is recommended as a control antibody for monitoring of CENP-K 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 CENP-K gene expression knockdown using RT-PCR Primer: CENP-K (h)-PR: sc-91868-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.