SANTA CRUZ BIOTECHNOLOGY, INC.

CENP-H siRNA (h): sc-37565

BACKGROUND

A replicated chromosome includes two kinetochores that control chromosome segregation during mitosis. Both centromere proteins CENP-B and CENP-H are contained in the centromeric heterochromatin between kinetochores, and are involved in maintaining sister chromatid cohesion. The highly dispersed CENP-B promotes and maintains the joining of DNA satellites in the centromere. CENP-B targets centromeric α-DNA and protects it from digestion by nucleases as well as preventing DNAse or restriction enzyme digestion from affecting the morphology of centromeres. CENP-H contains a coiled-coil structure and a nuclear localization signal. CENP-H is specifically and constitutively localized to kinetochores and plays a role in the organization and function of kinetochores throughout the cell cycle.

REFERENCES

1. Cooke, C.A., et al. 1990. CENP-B: a major human centromere protein located beneath the kinetochore. J. Cell Biol. 110: 1475-1488.
2. Rieder, C.L., et al. 1998. The vertebrate cell kinetochore and its roles during mitosis. Trends Cell Biol. 8: 310-318.
3. Barbosa-Cisneros, O., et al. 1998. Localization of the centromere protein CENP-B using scleroderma sera and evidence for a role in centromere survival. Rev. Rhum. Engl. Ed 65: 15-20.
4. Sugata, N., et al. 1999. Characterization of a novel kinetochore protein, CENP-H. J. Biol. Chem. 274: 27343-27346.
5. Choo, K.H. 2000. Centromerization. Trends Cell Biol. 10: 182-188.
6. 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.
7. Orthaus, S., et al. 2006. RNAi knockdown of human kinetochore protein CENP-H. Biochem. Biophys. Res. Commun. 348: 36-46.
8. Shigeishi, H., et al. 2006. Increased expression of CENP-H gene in human oral squamous cell carcinomas harboring high-proliferative activity. Oncol. Rep. 16: 1071-1075.

CHROMOSOMAL LOCATION

Genetic locus: CENPH (human) mapping to 5q13.2.

PRODUCT

CENP-H 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-H shRNA Plasmid (h): sc-37565-SH and CENP-H shRNA (h) Lentiviral Particles: sc-37565-V as alternate gene silencing products.

For independent verification of CENP-H (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-37565A, sc-37565B and sc-37565C.

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-H siRNA (h) is recommended for the inhibition of CENP-H 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-H (G-9): sc-365222 is recommended as a control antibody for monitoring of CENP-H 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-H gene expression knockdown using RT-PCR Primer: CENP-H (h)-PR: sc-37565-PR (20 µl). Annealing temperature for th e 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.