

CENP-A siRNA (h): sc-37555

BACKGROUND

A replicated chromosome includes two kinetochores that control chromosome segregation during mitosis. Centromere protein-A (CENP-A) is a Histone H3-like protein that contains a C-terminal H3-like domain, required for centromere localization of CENP-A, and an antigenic N-terminal domain. CENP-A, originally isolated from HeLa cells, is essential for kinetochore targeting of CENP-C. In the presence of DNA, CENP-A forms an octameric complex with Histones H4, H2A and H2B. CENP-A specifically localizes to active centromeres and is a component of specialized centromeric nucleosomes, on which kinetochores are assembled. CENP-A is essential for nucleosomal packaging of centromeric DNA at interphase and functions as a centromere formation marker on the chromosome.

REFERENCES

1. Rieder, C.L., et al. 1998. The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* 8: 310-318.
2. Choo, K.H. 2000. Centromerization. *Trends Cell Biol.* 10: 182-188.
3. Muro, Y., et al. 2000. Autoepitopes on autoantigen centromere protein-A (CENP-A) are restricted to the N-terminal region, which has no homology with Histone H3. *Clin. Exp. Immunol.* 120: 218-223.
4. Howman, E.V., et al. 2000. Early disruption of centromeric chromatin organization in centromere protein A (CENP-A) null mice. *Proc. Natl. Acad. Sci. USA* 97: 1148-1153.
5. Yoda, K., et al. 2000. Human centromere protein A (CENP-A) can replace Histone H3 in nucleosome reconstitution *in vitro*. *Proc. Natl. Acad. Sci. USA* 97: 7266-7271.
6. Black, B.E., et al. 2007. Centromere identity maintained by nucleosomes assembled with Histone H3 containing the CENP-A targeting domain. *Mol. Cell* 25: 309-322.
7. Okamoto, Y., et al. 2007. A minimal CENP-A core is required for nucleation and maintenance of a functional human centromere. *EMBO J.* 26: 1279-1291.
8. Maddox, P.S., et al. 2007. Functional genomics identifies a Myb domain-containing protein family required for assembly of CENP-A chromatin. *J. Cell Biol.* 176: 757-763.

CHROMOSOMAL LOCATION

Genetic locus: CENPA (human) mapping to 2p23.3.

PRODUCT

CENP-A 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 CENP-A shRNA Plasmid (h): sc-37555-SH and CENP-A shRNA (h) Lentiviral Particles: sc-37555-V as alternate gene silencing products.

For independent verification of CENP-A (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-37555A, sc-37555B and sc-37555C.

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

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

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CENP-A gene expression knockdown using RT-PCR Primer: CENP-A (h)-PR: sc-37555-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Petsalaki, E. and Zachos, G. 2016. Clks 1, 2 and 4 prevent chromatin breakage by regulating the Aurora B-dependent abscission checkpoint. *Nat. Commun.* 7: 11451.

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.