SANTA CRUZ BIOTECHNOLOGY, INC.

INCENP siRNA (m): sc-60849

BACKGROUND

A replicated chromosome includes two kinetochores that control chromosome segregation during mitosis. The centromere proteins CENP-A, CENP-B, CENP-C, CENP-E, CENP-F (also designated mitosin), CENP-H and INCENP are kinetochore proteins that are involved in mitotic events. The centromere proteins are expressed at different levels throughout the cell cycle and are involved in the formation of the centromere and the organization and function of the kinetochore. INCENP, which also is designated inner centromere protein, is a chromosomal passenger protein that is crucial for chromosome segregation. During mitosis it is also required for cytokinesis onset. This protein, which can form a homodimer or a heterodimer, binds directly to microtubules and interacts with AURKB, AURKC, CBX3 and β Tubulin. This nuclear protein localizes to the mitotic spindle, methaphase chromosomes and during anaphase, to the equatorial cortex.

REFERENCES

1. Ainsztein, A.M., et al. 1998. INCENP centromere and spindle targeting: identification of essential conserved motifs and involvement of heterochromatin protein HP1. J. Cell Biol. 143: 1763-1774.
2. Wheatley, S.P., et al. 2001. INCENP binds directly to Tubulin and requires dynamic microtubules to target to the cleavage furrow. Exp. Cell Res. 262: 122-127.
3. Li, X., et al. 2004. Direct association with inner centromere protein (INCENP) activates the novel chromosomal passenger protein, Aurora C. J. Biol. Chem. 279: 47201-47211.
4. Vernos, I., et al. 2004. The chromosomal passenger complex takes center stage during mitosis. Dev. Cell 7: 145-146.
5. Sessa, F., et al. 2005. Mechanism of Aurora B activation by INCENP and inhibition by hesperadin. Mol. Cell 18: 379-391.
6. Zhu, C., et al. 2005. Recruitment of MKLP1 to the spindle midzone/midbody by INCENP is essential for midbody formation and completion of cytokinesis in human cells. Biochem. J. 389: 373-381.
7. Nousiainen, M., et al. 2006. Phosphoproteome analysis of the human mitotic spindle. Proc. Natl. Acad. Sci. USA 103: 5391-5396.

CHROMOSOMAL LOCATION

Genetic locus: Incenp (mouse) mapping to 19 A.

PRODUCT

INCENP siRNA (m) 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 INCENP shRNA Plasmid (m): sc-60849-SH and INCENP shRNA (m) Lentiviral Particles: sc-60849-V as alternate gene silencing products.

For independent verification of INCENP (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60849A, sc-60849B and sc-60849C.

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

INCENP siRNA (m) is recommended for the inhibition of INCENP expression in mouse 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

INCENP (B-4): sc-376514 is recommended as a control antibody for monitoring of INCENP 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 INCENP gene expression knockdown using RT-PCR Primer: INCENP (m)-PR: sc-60849-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.