



CENP-E siRNA (h): sc-37561

BACKGROUND

A replicated chromosome includes two kinetochores that control chromosome segregation during mitosis. Centromere Protein E, CENP-E, is a kinetochore motor protein that specifies kinetochore binding in mitosis. Both CENP-E and CENP-F are expressed during mitosis where they mediate the G₂ to M phase checkpoint. CENP-E is also expressed in high levels during meiosis I and meiosis II where it localizes to the fibrous corona and outer plate of kinetochores on the meiotic chromosomes. CENP-E colocalizes with hBUBR1, a BUB-related kinase until mid-anaphase. After the first polar body emission, CENP-E localizes to the spindle-midzone, separating from hBUBR1 after mid-anaphase.

REFERENCES

1. Rieder, C.L., et al. 1998. The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* 8: 310-318.
2. Chan, G.K., et al. 1998. Characterization of the kinetochore binding domain of CENP-E reveals interactions with the kinetochore proteins CENP-F and hBUBR1. *J. Cell Biol.* 143: 49-63.
3. Ashar, H.R., et al. 2000. Farnesyl transferase inhibitors block the farnesylation of CENP-E and CENP-F and alter the association of CENP-E with the microtubules. *J. Biol. Chem.* 275: 30451-30457.
4. Lee, J., et al. 2000. Specific regulation of CENP-E and kinetochores during meiosis I/meiosis II transition in pig oocytes. *Mol. Reprod. Dev.* 56: 51-62.
5. Choo, K.H. 2000. Centromerization. *Trends Cell Biol.* 10: 182-188.

CHROMOSOMAL LOCATION

Genetic locus: CENPE (human) mapping to 4q24.

PRODUCT

CENP-E 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-E shRNA Plasmid (h): sc-37561-SH and CENP-E shRNA (h) Lentiviral Particles: sc-37561-V as alternate gene silencing products.

For independent verification of CENP-E (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-37561A, sc-37561B and sc-37561C.

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

GENE EXPRESSION MONITORING

CENP-E (C-5): sc-376685 is recommended as a control antibody for monitoring of CENP-E 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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-E gene expression knockdown using RT-PCR Primer: CENP-E (h)-PR: sc-37561-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. Xiao, T., et al. 2015. CTCF recruits centromeric protein CENP-E to the pericentromeric/centromeric regions of chromosomes through unusual CTCF-binding sites. *Cell Rep.* 12: 1704-1714.

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.