SANTA CRUZ BIOTECHNOLOGY, INC.

CEP164 siRNA (h): sc-96897

BACKGROUND

Centrosomes are the major microtubule-organizing centers of mammalian cells. They are composed of a centriole pair and surrounding microtubule-nucleating material termed pericentriolar material (PCM). Bipolar mitotic spindle assembly relies on two intertwined processes: centriole duplication and centrosome maturation. Failure to properly orchestrate centrosome duplication and maturation is subsequently linked to spindle defects, which can result in aneuploidy and promote cancer progression. CEP164 (Centrosomal protein of 164 kDa) is a 1,460 amino acid protein that plays a critical role in G_2/M checkpoint and nuclear divisions by maintaining the formation of primary cilia. CEP164 is required for proper phosphorylation of Chk1, Chk2, Histone H2A and RPA and is therefore an essential player in the DNA-damage-activated ATR/ATM signaling cascade. Localized to the centrioles throughout mitosis, CEP164 also is required for the maintenance of genomic stability and chromosomal segregation. There are two isoforms of CEP164 that are produced as a result of alternative splicing events.

REFERENCES

1. Bornens, M. 2002. Centrosome composition and microtubule anchoring mechanisms. Curr. Opin. Cell Biol. 14: 25-34.
2. Uetake, Y., et al. 2004. Interaction of Cep135 with a p50 dynactin subunit in mammalian centrosomes. Cell Motil. Cytoskeleton 58: 53-66.
3. Manneville, J.B., et al. 2006. Positioning centrosomes and spindle poles: looking at the periphery to find the centre. Biol. Cell 98: 557-565.
4. Graser, S., et al. 2007. Cep164, a novel centriole appendage protein required for primary cilium formation. J. Cell Biol. 179: 321-330.
5. Sivasubramaniam, S., et al. 2008. Cep164 is a mediator protein required for the maintenance of genomic stability through modulation of MDC1, RPA, and CHK1. Genes Dev. 22: 587-600.
6. Pan, Y.R., et al. 2009. UV-dependent interaction between Cep164 and XPA mediates localization of Cep164 at sites of DNA damage and UV sensitivity. Cell Cycle. 8: 655-664.

CHROMOSOMAL LOCATION

Genetic locus: CEP164 (human) mapping to 11q23.3.

PRODUCT

CEP164 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 CEP164 shRNA Plasmid (h): sc-96897-SH and CEP164 shRNA (h) Lentiviral Particles: sc-96897-V as alternate gene silencing products.

For independent verification of CEP164 (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-96897A, sc-96897B and sc-96897C.

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

CEP164 siRNA (h) is recommended for the inhibition of CEP164 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

CEP164 (E-9): sc-515403 is recommended as a control antibody for monitoring of CEP164 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 CEP164 gene expression knockdown using RT-PCR Primer: CEP164 (h)-PR: sc-96897-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.