

# Hec1 siRNA (h): sc-37612

## BACKGROUND

Highly expressed in cancer (Hec1) is a coiled-coil-enriched protein expressed abundantly in the S and M phases of rapidly dividing cells where it localizes to the kinetochores. Hec1 is involved in spindle checkpoint signaling. Hec1 is not expressed in terminal differentiated cells. Hec1 is expressed in tissues with high mitotic rates including testis, spleen and thymus. Hec1 is also found in the late S to M phases of bladder carcinoma cells. In dividing cells, Hec1 is required for the recruitment of Mps1 kinase and MAD1/MAD2 complexes to the kinetochores. The phosphorylation of Hec1 on Serine 165 by Nek2 is essential for faithful chromosome segregation. The binding of retinoblastoma protein to Hec1 also increases the fidelity of chromosomal segregation.

## REFERENCES

1. Chen, Y., Riley, D.J., Chen, P.L. and Lee, W.H. 1997. HEC, a novel nuclear protein rich in leucine heptad repeats specifically involved in mitosis. *Mol. Cell. Biol.* 17: 6049-6056.
2. Zheng, L., Chen, Y., Riley, D.J., Chen, P.L. and Lee, W.H. 2000. Retinoblastoma protein enhances the fidelity of chromosome segregation mediated by hsHec1p. *Mol. Cell. Biol.* 20: 3529-3537.
3. Martin-Lluesma, S., Stucke, V.M. and Nigg, E.A. 2002. Role of Hec1 in spindle checkpoint signaling and kinetochore recruitment of MAD1/MAD2. *Science* 297: 2267-2270.
4. Chen, Y., Riley, D.J., Zheng, L., Chen, P.L. and Lee, W.H. 2002. Phosphorylation of the mitotic regulator protein Hec1 by Nek2 kinase is essential for faithful chromosome segregation. *J. Biol. Chem.* 277: 49408-49416.
5. LocusLink Report (LocusID: 10403) <http://www.ncbi.nlm.nih.gov/LocusLink>

## CHROMOSOMAL LOCATION

Genetic locus: NDC80 (human) mapping to 18p11.32.

## PRODUCT

Hec1 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Hec1 shRNA Plasmid (h): sc-37612-SH and Hec1 shRNA (h) Lentiviral Particles: sc-37612-V as alternate gene silencing products.

For independent verification of Hec1 (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-37612A, sc-37612B and sc-37612C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

Hec1 siRNA (h) is recommended for the inhibition of Hec1 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  $\mu$ M in 66  $\mu$ 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

Hec1 (C-11): sc-515550 is recommended as a control antibody for monitoring of Hec1 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Hec1 gene expression knockdown using RT-PCR Primer: Hec1 (h)-PR: sc-37612-PR (20  $\mu$ l). 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](http://www.scbt.com) for detailed protocols and support products.