SANTA CRUZ BIOTECHNOLOGY, INC.

Herc3 siRNA (h): sc-89133

BACKGROUND

Herc3 (HECT domain and RCC1-like domain-containing protein 3) is a 1,050 amino acid protein that localizes to both the cytoplasm and to vesicular-like structures. Involved in protein degradation pathways, Herc3 functions as an E3 ubiquitin ligase that accepts a ubiquitin residue from an E2 ubiquitin-conjugating enzyme and immediately transfers that residue to a protein that is targeted for degradation. Herc3, like other members of the Herc family, contains one HECT (E6AP-type E3 ubiquitin-protein ligase) domain and seven RCC1 repeats through which is conveys its E3 ubiquitin ligase activity. Upon ubiquitination, Herc3 is targeted for proteasomal degradation.

REFERENCES

1. Nomura, N., et al. 1994. Prediction of the coding sequences of unidentified human genes. I. The coding sequences of 40 new genes (KIAA0001-KIAA0040) deduced by analysis of randomly sampled cDNA clones from human immature myeloid cell line KG-1. DNA Res. 1: 27-35.
2. Cruz, C., et al. 1999. Assignment of the human P532 gene (Herc1) to chromosome 15q22 by fluorescence *in situ* hybridization. Cytogenet. Cell Genet. 86: 68-69.
3. Cruz, C., et al. 1999. The human Herc3 gene maps to chromosome 4q21 by fluorescence *in situ* hybridization. Cytogenet. Cell Genet. 87: 263-264.
4. Online Mendelian Inheritance in Man, OMIM™. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 605200. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Hochrainer, K., et al. 2005. The human Herc family of ubiquitin ligases: novel members, genomic organization, expression profiling, and evolutionary aspects. Genomics 85: 153-164.

CHROMOSOMAL LOCATION

Genetic locus: HERC3 (human) mapping to 4q22.1.

PRODUCT

Herc3 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 Herc3 shRNA Plasmid (h): sc-89133-SH and Herc3 shRNA (h) Lentiviral Particles: sc-89133-V as alternate gene silencing products.

For independent verification of Herc3 (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-89133A, sc-89133B and sc-89133C.

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.

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

Herc3 siRNA (h) is recommended for the inhibition of Herc3 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

Herc3 (37.2): sc-100720 is recommended as a control antibody for monitoring of Herc3 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 Herc3 gene expression knockdown using RT-PCR Primer: Herc3 (h)-PR: sc-89133-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.