SANTA CRUZ BIOTECHNOLOGY, INC.

Herc1 siRNA (h): sc-90102

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The Power to Question

BACKGROUND

One of the largest human proteins, Herc1, also designated p532 or p619, is a 4861 amino acid protein that contains a variety of functional domains, including WD repeats, RCC1 repeats, beta-repeat domains and one HECT domain. Herc1 is ubiquitously expressed in human and mouse tissues and is overexpressed in several human tumor cell lines. It localizes to the cytosol and the Golgi apparatus, where it acts as a guanine nucleotide exchange factor on ARF1 and Rab proteins to mediate membrane trafficking. Herc1 also is involved in proliferation and growth through its interactions with clathrin, M2-pyruvate kinase and TSC2 proteins. Mutations in the TSC2 protein allow for binding to Herc1 in the presence of TSC1, which destabilizes the TSC2 protein. This results in tuberous sclerosis complex (TSC), an autosomal dominant disease characterized by hamartoma formation in various organs.

REFERENCES

1. Rosa, J.L., et al. 1996. p619, a giant protein related to the chromosome condensation regulator RCC1, stimulates guanine nucleotide exchange on ARF1 and Rab proteins. EMBO J. 15: 4262-4273.
2. Rosa, J.L., et al. 1997. A giant protein that stimulates guanine nucleotide exchange on ARF1 and Rab proteins forms a cytosolic ternary complex with clathrin and Hsp70. Oncogene 15: 1-6.
3. 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.
4. Garcia-Gonzalo, F.R., et al. 2003. Interaction between HERC1 and M2-type pyruvate kinase. FEBS Lett. 539: 78-84.
5. Garcia-Gonzalo, F.R., et al. 2004. The giant protein HERC1 is recruited to aluminum fluoride-induced Actin-rich surface protrusions in HeLa cells. FEBS Lett. 559: 77-83.
6. Garcia-Gonzalo, F.R., et al. 2005. Requirement of phosphatidylinositol-4,5-bisphosphate for HERC1-mediated guanine nucleotide release from ARF proteins. FEBS Lett. 579: 343-348.
7. Chong-Kopera, H., et al. 2006. TSC1 stabilizes TSC2 by inhibiting the interaction between TSC2 and the HERC1 ubiquitin ligase. J. Biol. Chem. 281: 8313-8316.

CHROMOSOMAL LOCATION

Genetic locus: HERC1 (human) mapping to 15q22.31.

PRODUCT

Herc1 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 Herc1 shRNA Plasmid (h): sc-90102-SH and Herc1 shRNA (h) Lentiviral Particles: sc-90102-V as alternate gene silencing products.

For independent verification of Herc1 (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-90102A, sc-90102B and sc-90102C.

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

Herc1 siRNA (h) is recommended for the inhibition of Herc1 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

Herc1 (E-12): sc-393950 is recommended as a control antibody for monitoring of Herc1 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 Herc1 gene expression knockdown using RT-PCR Primer: Herc1 (h)-PR: sc-90102-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.