SANTA CRUZ BIOTECHNOLOGY, INC.

Herc2 siRNA (h): sc-89942

BACKGROUND

Ubiquitination is an important mechanism through which three classes of enzymes act in concert to target short-lived or abnormal proteins for destruction. The three classes of enzymes involved in ubiquitination are the ubiquitin-activating enzymes (E1s), the ubiquitin-conjugating enzymes (E2s) and the ubiquitin-protein ligases (E3s). Herc2 (HECT domain and RLD 2), also known as jdf2, p528 or SHEP1, is a 4,834 amino acid protein that contains a variety of functional domains, including WD repeats, RCC1 repeats, HECT domains and ZZ-type zinc fingers. Involved in the pathway of protein modification, Herc2 is thought to function as an E3 ubiquitin-protein ligase that accepts ubiquitin (in the form of a thioester) from an E2 ubiquitin-conjugating enzyme and transfers that ubiquitin residue to substrates targeted for degradation. Variations in the Herc2 gene are associated with skin/hair/eye pigmentation variability type 1 (SHEP1), a allelic modification that affects hair, eye and skin color.

REFERENCES

1. Ji, Y., et al. 2000. Structure of the highly conserved Herc2 gene and of multiple partially duplicated paralogs in human. Genome Res. 10: 319-329.
2. Kayser, M., et al. 2008. Three genome-wide association studies and a linkage analysis identify Herc2 as a human iris color gene. Am. J. Hum. Genet. 82: 411-423.
3. Sturm, R.A., et al. 2008. A single SNP in an evolutionary conserved region within intron 86 of the Herc2 gene determines human blue-brown eye color. Am. J. Hum. Genet. 82: 424-431.
4. Eiberg, H., et al. 2008. Blue eye color in humans may be caused by a perfectly associated founder mutation in a regulatory element located within the Herc2 gene inhibiting OCA2 expression. Hum. Genet. 123: 177-187.

CHROMOSOMAL LOCATION

Genetic locus: HERC2 (human) mapping to 15q13.1.

PRODUCT

Herc2 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 Herc2 shRNA Plasmid (h): sc-89942-SH and Herc2 shRNA (h) Lentiviral Particles: sc-89942-V as alternate gene silencing products.

For independent verification of Herc2 (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-89942A, sc-89942B and sc-89942C.

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

Herc2 siRNA (h) is recommended for the inhibition of Herc2 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

Herc2 (A-5): sc-515891 is recommended as a control antibody for monitoring of Herc2 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 Herc2 gene expression knockdown using RT-PCR Primer: Herc2 (h)-PR: sc-89942-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.