SANTA CRUZ BIOTECHNOLOGY, INC.

HECTD1 siRNA (m): sc-145928

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

HECT (homologous to the E6-AP carboxyl-terminus) proteins are a large group of E3 ubiquitin-ligases that play a role in the specificity and selectivity of ubiquitylation. The human genome encodes at least 20 different HECT domain proteins, which are grouped into 2 classes based on their E2 specificity. HECT enzymes also regulate the trafficking of many receptors, transporters, viral proteins and channels. Since HECT proteins are involved in the degradation of vital tumor suppressor molecules, it is theorized that some may contribute to tumorigenesis. HECTD1 (HECT domain containing 1), also known as opm, is a 2,618 amino acid protein that is ubiquitously expressed throughout early development of the embryo. Containing four ANK repeats, one HECT (E6AP-type E3 ubiquitin-protein ligase) domain and a MIB/HERC2 domain, HECTD1 interacts with IGSF1 and may be involved in protein ubiquitination.

REFERENCES

1. Schwarz, S.E., et al. 1998. Characterization of human hect domain family members and their interaction with UbcH5 and UbcH7. J. Biol. Chem. 273: 12148-12154.
2. Scheffner, M. and Staub, O. 2007. HECT E3s and human disease. BMC Biochem. 8: S6.
3. Kee, Y. and Huibregtse, J.M. 2007. Regulation of catalytic activities of HECT ubiquitin ligases. Biochem. Biophys. Res. Commun. 354: 329-333.
4. Zohn, I.E., et al. 2007. The HECTD1 ubiquitin ligase is required for development of the head mesenchyme and neural tube closure. Dev. Biol. 306: 208-221.

CHROMOSOMAL LOCATION

Genetic locus: Hectd1 (mouse) mapping to 12 C1.

PRODUCT

HECTD1 siRNA (m) 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 HECTD1 shRNA Plasmid (m): sc-145928-SH and HECTD1 shRNA (m) Lentiviral Particles: sc-145928-V as alternate gene silencing products.

For independent verification of HECTD1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-145928A, sc-145928B and sc-145928C.

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

HECTD1 siRNA (m) is recommended for the inhibition of HECTD1 expression in mouse 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

HECTD1 (1E10): sc-517169 is recommended as a control antibody for monitoring of HECTD1 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 HECTD1 gene expression knockdown using RT-PCR Primer: HECTD1 (m)-PR: sc-145928-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Shen, Y., et al. 2022. Ursodeoxycholic acid reduces antitumor immunosuppression by inducing CHIP-mediated TGF-β degradation. Nat. Commun. 13: 3419.

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.