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HECTD3 siRNA (m): sc-145930

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. HECTD3 (HECT domain-containing protein 3) is a 861 amino acid E3 ubiquitin-ligase that characteristically accepts ubiquitin from an E2 ubiquitin-conjugating enzyme and directly transfers the ubiquitin to targeted substrates. HECTD3 directly binds Tara, a protein that plays a role in organization of the actin cytoskeleton and cell cycle regulation. Overexpression of HECTD3 enhances ubiquitination of Tara, suggesting that HECTD3 may facilitate cell cycle progression by regulating Tara degradation. There are two isoforms of HECTD3 that are produced as a result of alternative splicing events.

REFERENCES

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- Zhang, L., Kang, L., Bond, W. and Zhang, N. 2009. Interaction between syntaxin 8 and HECTd3, a HECT domain ligase. *Cell. Mol. Neurobiol.* 29: 115-121.

CHROMOSOMAL LOCATION

Genetic locus: Hectd3 (mouse) mapping to 4 D1.

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.

PRODUCT

HECTD3 siRNA (m) is a pool of 2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HECTD3 shRNA Plasmid (m): sc-145930-SH and HECTD3 shRNA (m) Lentiviral Particles: sc-145930-V as alternate gene silencing products.

For independent verification of HECTD3 (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-145930A and sc-145930B.

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

APPLICATIONS

HECTD3 siRNA (m) is recommended for the inhibition of HECTD3 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 μ M in 66 μ 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HECTD3 gene expression knockdown using RT-PCR Primer: HECTD3 (m)-PR: sc-145930-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.