SANTA CRUZ BIOTECHNOLOGY, INC.

EDD siRNA (m): sc-143292

BACKGROUND

EDD (for E3 identified by differential display) is a progestin-regulated gene that was isolated from T-47D human breast cancer cells. Based on sequence homology, EDD appears to be a human homolog of the *Drosophila* hyperplastic discs (hyd) gene, a tumor suppressor gene that is required for control of imaginal disc growth. EDD contains a HECT domain in the carboxy terminus. HECT domain-containing proteins function as ubiquitin-protein ligases, or E3 enzymes. EDD has been shown to bind to ubiquitin, and like other HECT family proteins, may function as an E3 ubiquitin-protein ligase.

REFERENCES

1. Mansfield, E., et al. 1994. Genetic and molecular analysis of hyperplastic discs, a gene whose product is required for regulation of cell proliferation in *Drosophila melanogaster* imaginal discs and germ cells. Dev. Biol. 165: 507-526.
2. Huibregtse, J.M., et al. 1995. A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. Proc. Natl. Acad. Sci. USA 92: 5249.
3. Huibregtse, J.M., et al. 1997. The large subunit of RNA polymerase II is a substrate of the Rsp5 ubiquitin-protein ligase. Proc. Natl. Acad. Sci. USA 94: 3656-3661.
4. Hatakeyama, S., et al. 1997. Subcellular localization and ubiquitin-conjugating enzyme (E2) interactions of mammalian HECT family ubiquitin protein ligases. J. Biol. Chem. 272: 15085-15092.
5. Callaghan, M.J., et al. 1998. Identification of a human HECT family protein with homology to the *Drosophila* tumor suppressor gene hyperplastic discs. Oncogene 17: 3479-3491.

CHROMOSOMAL LOCATION

Genetic locus: Ubr5 (mouse) mapping to 15 B3.1.

PRODUCT

EDD siRNA (m) is a target-specific 19-25 nt siRNA 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 EDD shRNA Plasmid (m): sc-143292-SH and EDD shRNA (m) Lentiviral Particles: sc-143292-V as alternate gene silencing 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

EDD siRNA (m) is recommended for the inhibition of EDD 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

EDD (B-11): sc-515494 is recommended as a control antibody for monitoring of EDD 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 EDD gene expression knockdown using RT-PCR Primer: EDD (m)-PR: sc-143292-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.