SANTA CRUZ BIOTECHNOLOGY, INC.

EED siRNA (h): sc-37823

BACKGROUND

The transcriptional repressing Polycomb-group (PcG) and transcriptional activating trithorax-group (trxG) genes of *Drosophila* are part of a cellular memory system responsible for the stable inheritance of gene activity. PcG proteins assemble into multimeric protein complexes, which are involved in maintaining the transcriptional repressive state of genes over successive cell generations. EED (embryonic ectoderm development) is the human homolog of Eed, a murine PcG gene homologous to the *Drosophila* homeotic gene, extra sex combs. The human EED protein is 99.5% identical to the mouse EED protein and contains seven WD repeats, which are involved in protein-protein interactions. There are two human EED transcripts that contain a putative 407-nucleotide-long intron and give rise to two HEED protein isoforms, 535 and 494 amino acids in length. EED interacts in a highly specific manner, both *in vitro* and *in vivo,* with histone deacetylase (HDAC) proteins.

REFERENCES

1. Denisenko, O., et al. 1998. Point mutations in the WD40 domain of Eed block its interaction with Ezh2. Mol. Cell. Biol. 18: 5634-5642.
2. Sewalt, R.G., et al. 1998. Characterization of interactions between the mammalian polycomb-group proteins Enx1/EZH2 and EED suggests the existence of different mammalian polycomb-group protein complexes. Mol. Cell. Biol. 18: 3586-3595.
3. van der Vlag, J., et al. 1999. Transcriptional repression mediated by the human polycomb-group protein EED involves histone deacetylation. Nat. Genet. 23: 474-478.
4. Peytavi, R., et al. 1999. HEED, the product of the human homolog of the murine eed gene, binds to the matrix protein of HIV-1. J. Biol. Chem. 274: 1635-1645.
5. Wang, J., et al. 2001. Imprinted X inactivation maintained by a mouse Polycomb group gene. Nat. Genet. 28: 371-375.
6. Online Mendelian Inheritance in Man, OMIM™. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 605984. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: EED (human) mapping to 11q14.2.

PRODUCT

EED 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 EED shRNA Plasmid (h): sc-37823-SH and EED shRNA (h) Lentiviral Particles: sc-37823-V as alternate gene silencing products.

For independent verification of EED (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-37823A, sc-37823B and sc-37823C.

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

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

EED (3B12): sc-293203 is recommended as a control antibody for monitoring of EED 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 EED gene expression knockdown using RT-PCR Primer: EED (h)-PR: sc-37823-PR (20 µl, 470 bp). 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.