SANTA CRUZ BIOTECHNOLOGY, INC.

ESET siRNA (m): sc-45660

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The Power to Question

BACKGROUND

ERG-associated protein with SET domain (ESET), also designated Histone H3-K9 methyltransferase 4 or SET domain bifurcated 1, is a nuclear protein belonging to the histone-lysine methyltransferase family and to the Suvar3-9 subfamily. It is a highly conserved protein of 150 amino acids that has been implicated in chromatin structure modulation. ESET is excluded from cell nucleoli and areas of condensed chromatin and can associate with the non-pericentromeric regions of chromatin. The gene encoding for this protein, SETDB1, maps to chromosome 1q21. ESET is a histone methyltransferase, methylating Lys-9 of histone H3 and mutations within the SETDB1 gene abolishes its methyltransferase activity. This methylation acts as a tag for epigenetic transcriptional repression by rounding up HP1 proteins to methylated histones. ESET is widely expressed with highest levels found in testis.

REFERENCES

1. Nomura, N., et al. 1994. Prediction of the coding sequences of unidentified human genes. II. The coding sequences of 40 new genes (KIAA0041-KIAA0080) deduced by analysis of cDNA clones from human cell line KG-1. DNA Res. 1: 223-229.
2. Harte, P.J., et al. 1999. Assignment of a novel bifurcated SET domain gene, SETDB1, to human chromosome band 1q21 by *in situ* hybridization and radiation hybrids. Cytogenet. Cell Genet. 84: 83-86.
3. Yang, L., et al. 2002. Molecular cloning of ESET, a novel Histone H3-specific methyltransferase that interacts with ERG transcription factor. Oncogene 21: 148-152.
4. Wang, H., et al. 2003. mAM facilitates conversion by ESET of dimethyl to trimethyl Lysine 9 of Histone H3 to cause transcriptional repression. Mol. Cell 12: 475-487.
5. Baxter, J., et al. 2004. Histone hypomethylation is an indicator of epigenetic plasticity in quiescent lymphocytes. EMBO J. 23: 4462-4472.
6. Dodge, J.E., et al. 2004. Histone H3-K9 methyltransferase ESET is essential for early development. Mol. Cell. Biol. 24: 2478-2486.

CHROMOSOMAL LOCATION

Genetic locus: Setdb1 (mouse) mapping to 3 F2.1.

PRODUCT

ESET 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 ESET shRNA Plasmid (m): sc-45660-SH and ESET shRNA (m) Lentiviral Particles: sc-45660-V as alternate gene silencing products.

For independent verification of ESET (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-45660A, sc-45660B and sc-45660C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

ESET shRNA Plasmid (m) is recommended for the inhibition of ESET 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

ESET (A-1): sc-166621 is recommended as a control antibody for monitoring of ESET 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 ESET gene expression knockdown using RT-PCR Primer: ESET (m)-PR: sc-45660-PR (20 µl, 560 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.