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ESET shRNA (h) Lentiviral Particles: sc-45659-V

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 nonpericentromeric 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

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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.
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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 (human) mapping to 1q21.3.

PRODUCT

ESET shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ESET siRNA (h): sc-45659 and ESET shRNA Plasmid (h): sc-45659-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

ESET shRNA (h) Lentiviral Particles is recommended for the inhibition of ESET expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

ESET (A-7): sc-271553 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ESET gene expression knockdown using RT-PCR Primer: ESET (h)-PR: sc-45659-PR (20 μ l, 562 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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