



ETO siRNA (h): sc-35342

BACKGROUND

ETO and ETO-2, which are alternatively designated MTG8 and MTG16, respectively, are members of the ETO transcription factor family. These transcription factors are characterized by a zinc-finger domain and four conserved domains, of which domain II is required for dimerization between family members. ETO and ETO-2 may function to mediate interactions between DNA binding proteins and transcriptional regulators, such as N-CoR. Frequently, the t(8;21) translocation of ETO produces the AML-1/ETO oncoprotein, which consists of the first 177 amino acids of AML-1 and all but the first 30 amino acids of ETO. AML-1/ETO expression is observed in 12-15% of acute myelogenous, M2 subtype leukemias. The AML-1/ETO fusion proteins associate with multimeric N-CoR/mSin3/HDAC1 complexes, block differentiation and induce transcriptional repression by altering chromatin remodeling.

REFERENCES

1. Erickson, P.F., et al. 1994. The ETO portion of acute myeloid leukemia t(8;21) fusion transcript encodes a highly evolutionarily conserved, putative transcription factor. *Cancer Res.* 54: 1782-1786.
2. Erickson, P.F., et al. 1996. ETO and AML1 phosphoproteins are expressed in CD34⁺ hematopoietic progenitors: implications for t(8;21) leukemogenesis and monitoring residual disease. *Blood* 88: 1813-1823.
3. Wolford, J.K., et al. 1998. Structure and expression of the human MTG8/ETO gene. *Gene* 212: 103-109.
4. Wang, J., et al. 1998. ETO, fusion partner in t(8;21) acute myeloid leukemia, represses transcription by interaction with the human N-CoR/mSin3/HDAC1 complex. *Proc. Natl. Acad. Sci. USA* 95: 10860-10865.
5. Westendorf, J.J., et al. 1998. The t(8;21) fusion product, AML-1-ETO, associates with C/EBP- α , inhibits C/EBP- α -dependent transcription, and blocks granulocytic differentiation. *Mol. Cell. Biol.* 18: 322-333.
6. Davis, J.N., et al. 1999. ETO-2, a new member of the ETO-family of nuclear proteins. *Oncogene* 18: 1375-1383.
7. Wang, J., et al. 1999. Inhibitors of histone deacetylase relieve ETO-mediated repression and induce differentiation of AML1-ETO leukemia cells. *Cancer Res.* 59: 2766-2769.

CHROMOSOMAL LOCATION

Genetic locus: RUNX1T1 (human) mapping to 8q21.3.

PRODUCT

ETO 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ETO shRNA Plasmid (h): sc-35342-SH and ETO shRNA (h) Lentiviral Particles: sc-35342-V as alternate gene silencing products.

For independent verification of ETO (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-35342A, sc-35342B and sc-35342C.

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

ETO siRNA (h) is recommended for the inhibition of ETO 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 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.

GENE EXPRESSION MONITORING

ETO (3H11): sc-134335 is recommended as a control antibody for monitoring of ETO 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 ETO gene expression knockdown using RT-PCR Primer: ETO (h)-PR: sc-35342-PR (20 μ l, 449 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.