



ETO-2 siRNA (h): sc-35344

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: CBFA2T3 (human) mapping to 16q24.3.

PRODUCT

ETO-2 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-2 shRNA Plasmid (h): sc-35344-SH and ETO-2 shRNA (h) Lentiviral Particles: sc-35344-V as alternate gene silencing products.

For independent verification of ETO-2 (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-35344A, sc-35344B and sc-35344C.

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-2 siRNA (h) is recommended for the inhibition of ETO-2 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-2 (C-12): sc-373691 is recommended as a control antibody for monitoring of ETO-2 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-2 gene expression knockdown using RT-PCR Primer: ETO-2 (h)-PR: sc-35344-PR (20 μ l, 319 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.