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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

AF-10 siRNA (h): sc-43605

BACKGROUND

The nuclear protein AF-10 is one of several conserved transcription factors involved in the t(10;11) translocation in acute myeloid leukemia. The open reading frame of human AF-10 contains 1,027 amino acids, which are 90% identical to those of the mouse homolog, which contains 1,061 amino acids. AF-10 is primarily expressed in testis and is highly similar to AF-17.

REFERENCES

1. Chaplin, T., et al. 1995. A novel class of zinc finger/leucine zipper genes identified from the molecular cloning of the t(10;11) translocation in acute leukemia. *Blood* 85: 1435-1441.
2. Silliman, C.C., et al. 1998. Alternative splicing in wild-type AF-10 and CALM cDNAs and in AF-10-CALM and CALM-AF-10 fusion cDNAs produced by the t(10;11)(p13-14;q14-q21) suggests a potential role for truncated AF-10 polypeptides. *Leukemia* 12: 1404-1410.
3. Roll, P., et al. 2002. Molecular and fluorescence *in situ* hybridization analysis of a 10;11 rearrangement in a case of infant acute monocytic leukemia. *Cancer Genet. Cytogenet.* 135: 187-191.
4. Nakamura, T., et al. 2002. ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. *Mol. Cell* 10: 1119-1128.
5. Strausberg, R.L., et al. 2002. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc. Natl. Acad. Sci. USA* 99: 16899-16903.
6. Perrin, L., et al. 2003. The leucine zipper motif of the *Drosophila* AF-10 homologue can inhibit PRE-mediated repression: implications for leukemogenic activity of human MLL-AF-10 fusions. *Mol. Cell. Biol.* 23: 119-130.

CHROMOSOMAL LOCATION

Genetic locus: MLLT10 (human) mapping to 10p12.31.

PRODUCT

AF-10 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 AF-10 shRNA Plasmid (h): sc-43605-SH and AF-10 shRNA (h) Lentiviral Particles: sc-43605-V as alternate gene silencing products.

For independent verification of AF-10 (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-43605A, sc-43605B and sc-43605C.

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.

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

AF-10 siRNA (h) is recommended for the inhibition of AF-10 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

AF-10 (HAF10 9A5/2): sc-53156 is recommended as a control antibody for monitoring of AF-10 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 AF-10 gene expression knockdown using RT-PCR Primer: AF-10 (h)-PR: sc-43605-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.