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SANTA CRUZ BIOTECHNOLOGY, INC.

AF9 siRNA (m): sc-44794

BACKGROUND

The MLL (ALL-1, HRX) gene influences myelomonocytic differentiation, and different chromosomal translocations can result in a range of MLL fusion proteins that mediate leukemia. Frequent translocation partners of MLL include ELL, ENL, AF4, AF6 and AF9. ELL (elongation factor RNA polymerase II, Men) encodes an RNA polymerase II elongation factor that is implicated in t(11;19)(q23;p13.1) translocation in myeloid leukemias. AF9 (MLLT3, YEATS3) fusion with the MLL gene results in a t[(9;11)(p22;q23)] translocation, which is associated with *de novo* acute myelogenous leukemia (AML). ENL (MLLT1, LTG19, YEATS1, eleven-nineteen leukemia protein) is capable of activating transcription from synthetic reporter genes in both lymphoid and myeloid cells. The t[(11;19)(q23;p13)] translocation results in the MLL-ENL fusion protein, which is commonly found in infant acute leukemias of both the myeloid and lymphoid lineage.

REFERENCES

1. Ennas, M.G., et al. 1997. The human ALL-1/MLL/HRX antigen is predominantly localized in the nucleus of resting and proliferating peripheral blood mononuclear cells. Cancer Res. 57: 2035-2041.
2. Shilatifard, A. 1998. Factors regulating the transcriptional elongation activity of RNA polymerase II. FASEB J. 12: 1437-1446.
3. Shinobu, N., et al. 1999. Physical interaction and functional antagonism between the RNA polymerase II elongation factor ELL and p53. J. Biol. Chem. 274: 17003-17010.
4. Strissel, P.L., et al. 2000. DNA structural properties of AF9 are similar to MLL and could act as recombination hot spots resulting in MLL/AF9 translocations and leukemogenesis. Hum. Mol. Genet. 9: 1671-1679.
5. Horton, S.J., et al. 2005. Continuous MLL-ENL expression is necessary to establish a "Hox Code" and maintain immortalization of hematopoietic progenitor cells. Cancer 65: 9245-9252.
6. Murmann, A.E., et al. 2005. Local gene density predicts the spatial position of genetic loci in the interphase nucleus. Exp. Cell Res. 311: 14-26.

CHROMOSOMAL LOCATION

Genetic locus: Mllt3 (mouse) mapping to 4 C4.

PRODUCT

AF9 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 AF9 shRNA Plasmid (m): sc-44794-SH and AF9 shRNA (m) Lentiviral Particles: sc-44794-V as alternate gene silencing products.

For independent verification of AF9 (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-44794A, sc-44794B and sc-44794C.

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

AF9 siRNA (m) is recommended for the inhibition of AF9 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

AF9 (3C11): sc-293339 is recommended as a control antibody for monitoring of AF9 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 AF9 gene expression knockdown using RT-PCR Primer: AF9 (m)-PR: sc-44794-PR (20 µl, 476 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.