SANTA CRUZ BIOTECHNOLOGY, INC.

AFX1 siRNA (m): sc-29651

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The Power to Question

BACKGROUND

FKHR (for forkhead in rhabdomyosarcoma), FKHRL1 and AFX1 are members of a subfamily of the forkhead family of transcription factors. AFX1 expression is detected in a wide variety of tissues and, like other FKHR proteins, AFX1 contains a single forkhead domain and serine-proline-rich region, which mediate DNA binding. AFX1-mediated transcriptional activation is regulated by the serine/threonine kinase Akt1, which phosphorylates AFX1 and in turn, sequesters AFX1 in the cytosol, thereby blocking nuclear localization and DNA binding. Genetic mutations in FKHR genes, including the t(2;13) and t(1;3) translocations, are commonly found in alveolar rhabdo-myosarcomas. Additionally, the t(x;11) translocation of the AFX1 gene, which involves the fusion of a serine-proline-rich sequence of AFX1 to the carboxy terminus of a truncated MLL, results in acute lymphocytic leukemia.

REFERENCES

1. Corral, J., et al. 1993. Acute leukemias of different lineages have similar MLL gene fusions encoding related chimeric proteins resulting from chromosomal translocation. Proc. Natl. Acad. Sci. USA 90: 8538-8542.
2. Parry, P., et al. 1994. Cloning and characterization of the t(X;11) breakpoint from a leukemic cell line identify a new member of the forkhead gene family. Genes Chromosomes Cancer 11: 79-84.
3. Davis, R.J., et al. 1995. Structural characterization of the FKHR gene and its rearrangement in alveolar rhabdomyosarcoma. Hum. Mol. Genet. 4: 2355-2362.
4. Peters, U., et al. 1997. AFX1 and p54nrb: fine mapping, genomic structure, and exclusion as candidate genes of X-linked dystonia parkinsonism. Hum. Genet. 100: 569-572.
5. Anderson, M.J., et al. 1998. Cloning and characterization of three human forkhead genes that comprise an FKHR-like gene subfamily. Genomics 47: 187-199.
6. Kops, G.J., et al. 1999. Direct control of the forkhead transcription factor AFX by protein kinase B. Nature 398: 630-634.
7. Brunet, A., et al. 1999. Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor. Cell 96: 857-868.

CHROMOSOMAL LOCATION

Genetic locus: Mllt7 (mouse) mapping to X C3.

PRODUCT

AFX1 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 AFX1 shRNA Plasmid (m): sc-29651-SH and AFX1 shRNA (m) Lentiviral Particles: sc-29651-V as alternate gene silencing products.

For independent verification of AFX1 (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-29651A, sc-29651B and sc-29651C.

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

AFX1 siRNA (m) is recommended for the inhibition of AFX1 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

AFX1 (A-7): sc-373877 is recommended as a control antibody for monitoring of AFX1 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 AFX1 gene expression knockdown using RT-PCR Primer: AFX1 (m)-PR: sc-29651-PR (20 µl, 419 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Hori, Y.S., et al. 2013. Regulation of FOXOs and p53 by SIRT1 modulators under oxidative stress. PLoS ONE 8: e73875.

RESEARCH USE

For research use only, not for use in diagnostic procedures.