SANTA CRUZ BIOTECHNOLOGY, INC.

ALK siRNA (h): sc-40083

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The Power to Question

BACKGROUND

LTK, ALK and Ros have been identified as receptor tyrosine kinases having sequence similarity to the Insulin receptor subfamily of kinases. LTK (leukocyte tyrosine kinase) is expressed in murine B lymphocyte precursors and has also been found in forebrain neurons. ALK (anaplastic lymphoma kinase) is normally highly expressed, specifically in the nervous system. A truncated form containing the catalytic domain of ALK is expressed as the result of a translocation occuring in many non-Hodgkin's lymphomas. The c-Ros gene was originally identified in mutant form as an oncogene. Ros is normally expressed in a small number of epithelial cell types and may play a role in epithelial development.

REFERENCES

1. Birchmeier, C., et al. 1990. Characterization of Ros1 cDNA from a human glioblastoma cell line. Proc. Natl. Acad. Sci. USA 87: 4799-4803.
2. Haase, V.H., et al. 1991. Alternatively spliced LTK mRNA in neurons predicts a receptor with a larger putative extracellular domain. Oncogene 6: 2319-2325.
3. Morris, S.W., et al. 1994. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 263: 1281-1284.
4. Kanwar, Y.S., et al. 1995. Cloning of mouse c-Ros renal cDNA, its role in development a relationship to extracellular matrix glycoproteins. Kidney Int. 48: 1646-1659.
5. Sonnenberg-Riethmacher, E., et al. 1996. The c-Ros tyrosine kinase receptor controls regionalization and differentiation of epithelial cells in the epididymis. Genes Dev. 10: 1184-1193.

CHROMOSOMAL LOCATION

Genetic locus: ALK (human) mapping to 2p23.2.

PRODUCT

ALK 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ALK shRNA Plasmid (h): sc-40083-SH and ALK shRNA (h) Lentiviral Particles: sc-40083-V as alternate gene silencing products.

For independent verification of ALK (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-40083A, sc-40083B and sc-40083C.

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

ALK siRNA (h) is recommended for the inhibition of ALK 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 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

ALK (F-12): sc-398791 is recommended as a control antibody for monitoring of ALK 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 ALK gene expression knockdown using RT-PCR Primer: ALK (h)-PR: sc-40083-PR (20 µl, 495 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kim, H.R., et al. 2013. Epithelial-mesenchymal transition leads to crizotinib resistance in H2228 lung cancer cells with EML4-ALK translocation. Mol. Oncol. 7: 1093-1102.
2. Ji, C., et al. 2014. Induction of autophagy contributes to crizotinib resistance in ALK-positive lung cancer. Cancer Biol. Ther. 15: 570-577.
3. Fukuda, K., et al. 2019. Epithelial-to-mesenchymal transition is a mechanism of ALK inhibitor resistance in lung cancer independent of ALK mutation status. Cancer Res. 79: 1658-1670.
4. Tanimura, K., et al. 2022. HER3 activation contributes toward the emergence of ALK inhibitor-tolerant cells in ALK-rearranged lung cancer with mesenchymal features. NPJ Precis. Oncol. 6: 5.

RESEARCH USE

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