

DOT1L1 siRNA (h): sc-77174

BACKGROUND

DOT1L1, also known as DOT1L (DOT1-like, histone H3 methyltransferase), DOT1 or KMT4, is a 1739 amino acid homolog of the yeast DOT1 (disruptor of telomeric silencing-1) protein. Localized to the nucleus and highly expressed in testis, lung and kidney, DOT1L1 is a histone methyltransferase that transfers methyl groups from S-adenosyl-L-methionine to lysine residues on various substrates, such as nucleosomes or histones. While most histone methyltransferases contain a SET domain through which they confer their enzymatic activity, DOT1L1 does not contain this characteristic domain and is, therefore, thought to function through a different mechanism. DOT1L1 can bind with several MLL-fusion partners found in acute leukemia and, through this binding, can promote oncogenesis. Two isoforms of DOT1L1 are expressed due to alternative splicing events.

REFERENCES

1. Feng, Q., Wang, H., Ng, H.H., Erdjument-Bromage, H., Tempst, P., Struhl, K. and Zhang, Y. 2002. Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. *Curr. Biol.* 12: 1052-1058.
2. Min, J., Feng, Q., Li, Z., Zhang, Y. and Xu, R.M. 2003. Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. *Cell* 112: 711-723.
3. Okada, Y., Feng, Q., Lin, Y., Jiang, Q., Li, Y., Coffield, V.M., Su, L., Xu, G. and Zhang, Y. 2005. hDOT1L links histone methylation to leukemogenesis. *Cell* 121: 167-178.
4. Okada, Y., Jiang, Q., Lemieux, M., Jeannotte, L., Su, L. and Zhang, Y. 2006. Leukaemic transformation by CALM-AF10 involves upregulation of Hoxa5 by hDOT1L. *Nat. Cell Biol.* 8: 1017-1024.
5. Zhang, W., Xia, X., Reisenauer, M.R., Hemenway, C.S. and Kone, B.C. 2006. Dot1a-AF9 complex mediates histone H3 Lys-79 hypermethylation and repression of ENaC α in an aldosterone-sensitive manner. *J. Biol. Chem.* 281: 18059-18068.

CHROMOSOMAL LOCATION

Genetic locus: DOT1L (human) mapping to 19p13.3.

PRODUCT

DOT1L1 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 DOT1L1 shRNA Plasmid (h): sc-77174-SH and DOT1L1 shRNA (h) Lentiviral Particles: sc-77174-V as alternate gene silencing products.

For independent verification of DOT1L1 (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-77174A, sc-77174B and sc-77174C.

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

DOT1L1 siRNA (h) is recommended for the inhibition of DOT1L1 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

DOT1L1 (E-5): sc-374317 is recommended as a control antibody for monitoring of DOT1L1 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 DOT1L1 gene expression knockdown using RT-PCR Primer: DOT1L1 (h)-PR: sc-77174-PR (20 μ l, 572 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.