SANTA CRUZ BIOTECHNOLOGY, INC.

M33 siRNA (h): sc-38189

BACKGROUND

Polycomb group (PcG) proteins form multiprotein complexes and play a role in gene silencing and Hox gene regulation by altering chromatin structure during transcription. The PcG protein M33, also known as CBX2 or MOD2, controls the accessibility of retinoic acid response elements in the vicinity of HOX gene regulatory regions by direct and/or indirect mechanisms. MPc2 and MPc3 are PcG proteins that show structural similarity to M33 and, like M33, bind the PcG protein RING1 through a conserved c-box motif located in the C-terminus of RING1. Both M33 and Bmi-1 have an influence on positions effect variegation (PEV), which is the suppression of protein expression in a proportion of cells. M33 deficiency may cause sex reversal by interfering with steps upstream of the Y-chromosome-specific SRY gene. M33 may also be involved in two different pathologies: the campomelic syndrome, an inherited disorder, and neoplastic disorders linked to allele loss in this region.

REFERENCES

1. Gecz, J., et al. 1995. Assignment of a Polycomb-like chromobox gene (CBX2) to human chromosome 17q25. Genomics 26: 130-131.
2. Garcia, E., et al. 1999. RYBP, a new repressor protein that interacts with components of the mammalian Polycomb complex, and with the transcription factor YY1. EMBO J. 18: 3404-3418.
3. Bel-Vialar, S., et al. 2000. Altered retinoic acid sensitivity and temporal expression of HOX genes in polycomb-M33-deficient mice. Dev. Biol. 224: 238-249.
4. McMorrow, T., et al. 2000. Activation of the β globin locus by transcription factors and chromatin modifiers. EMBO J. 19: 4986-4996.
5. Bardos, J.I., et al. 2000. HPC3 is a new human polycomb orthologue that interacts and associates with RING1 and Bmi-1 and has transcriptional repression properties. J. Biol. Chem. 275: 28785-28792.
6. Hemenway, C.S., et al. 2001. The polycomb protein MPc3 interacts with AF9, an MLL fusion partner in t(9;11)(p22;q23) acute leukemias. Oncogene 20: 3798-3805.

CHROMOSOMAL LOCATION

Genetic locus: CBX2 (human) mapping to 17q25.3.

PRODUCT

M33 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 M33 shRNA Plasmid (h): sc-38189-SH and M33 shRNA (h) Lentiviral Particles: sc-38189-V as alternate gene silencing products.

For independent verification of M33 (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-38189A, sc-38189B and sc-38189C.

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

M33 siRNA (h) is recommended for the inhibition of M33 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

M33 (G-5): sc-515914 is recommended as a control antibody for monitoring of M33 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 M33 gene expression knockdown using RT-PCR Primer: M33 (h)-PR: sc-38189-PR (20 µl). 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.