SANTA CRUZ BIOTECHNOLOGY, INC.

CBX8 siRNA (m): sc-38196

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. CBX4 (chromobox homolog 4) and CBX8 (chromobox homolog 8), also known as PC2 or NBP16 and PC3 or RC1, respectively, 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. However, CBX8 has only been shown to bind RING1 *in vivo* with covalently modified forms of RING1. CBX8 also interacts with the carboxy-terminus of AF9, a transcriptional activator implicated in the development of acute lukemias. CBX8 acts as a long range transcriptional silencer when targeted to a reporter gene by a heterologous DNA-binding domain. The human MPc3 gene maps to chromosome 17q25.3 and encodes a 389 amino acid protein.

REFERENCES

1. Garcia, E., Marcos-Gutierrez, C., del Mar Lorente, M., Moreno, J.C. and Vidal, M. 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.
2. Bardos, J.I., Saurin, A.J., Tissot, C., Duprez, E. and Freemont, P.S. 2000. HPC3 is a new human polycomb orthologue that interacts and associates with RING1 and Bmi1 and has transcriptional repression properties. J. Biol. Chem. 275: 28785-28792.
3. Bel-Vialar, S., Core, N., Terranova, R., Goudot, V., Boned, A. and Djabali, M. 2000. Altered retinoic acid sensitivity and temporal expression of Hox genes in polycomb-M33-deficient mice. Dev. Biol. 224: 238-249.
4. Hemenway, C.S., de Erkenez, A.C. and Gould, G.C. 2001. The polycomb protein MPc3 interacts with AF9, an MLL fusion partner in t(9;11)(p22;q23) acute leukemias. Oncogene 20: 3798-3805.
5. LocusLink Report (LocusID: 57332). http://www.ncbi.nlm.nih.gov/LocusLink

CHROMOSOMAL LOCATION

Genetic locus: Cbx8 (mouse) mapping to 11 E2.

PRODUCT

CBX8 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 CBX8 shRNA Plasmid (m): sc-38196-SH and CBX8 shRNA (m) Lentiviral Particles: sc-38196-V as alternate gene silencing products.

For independent verification of CBX8 (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-38196A, sc-38196B and sc-38196C.

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 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

CBX8 siRNA (m) is recommended for the inhibition of CBX8 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

CBX8 (C-3): sc-374332 is recommended as a control antibody for monitoring of CBX8 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 CBX8 gene expression knockdown using RT-PCR Primer: CBX8 (m)-PR: sc-38196-PR (20 µl). 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.