SANTA CRUZ BIOTECHNOLOGY, INC.

ENX-2 siRNA (m): sc-38188

BACKGROUND

In *Drosophila*, the polycomb (PcG) gene family encodes chromatin proteins that are required for the repression of homeotic loci in embryonic development. PcG proteins work in conjunction with the trithorax-group (trxG) proteins, which activate homeobox gene expression during embryonic development. ENX-1, a mammalian homolog of the *Drosophila* gene enhancer of zeste, is a PcG protein that is ubiquitously expressed during early embryogenesis and becomes restricted to the central and peripheral nervous systems and sites of fetal hematopoiesis during later development. In the adult, ENX-1 is restricted to specific sites, including spleen, testis and placenta. ENX-2 is another mammalian homolog of the *Drosophila* gene enhancer of zeste and contains one SET domain. The gene for human ENX-2 maps to chromosome 17q21.2. ENX-2 expression is ubiquitous in adult and fetal tissue, where it may aid in maintaining heterochromatin stability.

REFERENCES

1. Goebl, M.G. 1991. The Bmi-1 and Mel-18 gene products define a new family of DNA-binding proteins involved in cell proliferation and tumorigenesis. Cell 66: 623.
2. Hobert, O., et al. 1996. Isolation and developmental expression analysis of ENX-1, a novel mouse polycomb group gene. Mech. Dev. 55: 171-184.
3. Abel, K.J., et al. 1996. Characterization of EZH1, a human homolog of *Drosophila* enhancer of zeste near BRCA1. Genomics 37: 161-171.
4. Laible, G., et al. 1997. Mammalian homologues of the polycomb-group gene enhancer of zeste mediate gene silencing in *Drosophila* heterochromatin and at *S. cerevisiae* telomeres. EMBO J. 16: 3219-3232.
5. van Lohuizen, M., et al. 1998. Interaction of mouse polycomb-group (Pc-G) proteins ENX-1 and ENX-2 with Eed: indication for separate Pc-G complexes. Mol. Cell. Biol. 18: 3572-3579.
6. Sewalt, R.G., et al. 1998. Characterization of interactions between the mammalian polycomb-group proteins ENX-1/EZH2 and EED suggests the existence of different mammalian polycomb-group protein complexes. Mol. Cell. Biol. 18: 3586-3595.

CHROMOSOMAL LOCATION

Genetic locus: Ezh1 (mouse) mapping to 11 D.

PRODUCT

ENX-2 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 ENX-2 shRNA Plasmid (m): sc-38188-SH and ENX-2 shRNA (m) Lentiviral Particles: sc-38188-V as alternate gene silencing products.

For independent verification of ENX-2 (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-38188A, sc-38188B and sc-38188C.

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

ENX-2 siRNA (m) is recommended for the inhibition of ENX-2 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

ENX-2 (H-4): sc-515817 is recommended as a control antibody for monitoring of ENX-2 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 ENX-2 gene expression knockdown using RT-PCR Primer: ENX-2 (m)-PR: sc-38188-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.