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Pbx 1/2/3/4 shRNA (h) Lentiviral Particles: sc-43999-V

BACKGROUND

Pbx 1, 2, 3 and 4 are members of the TALE (three amino acid loop extension) family of homeodomain-containing proteins. Human pre-B cell acute leukemias are frequently associated with a t(1;19)(q23;p13.3) chromosomal rearrangement, which creates a chimeric gene encoding a fusion between the E2A and Pbx 1 gene products. Pbx 2 and Pbx 3 share 92% and 94% respective identities with Pbx 1 over a 266 amino acid region flanking their homeobox domains, while all three proteins are quite divergent at their amino- and carboxy-termini. Two forms of Pbx 1 and Pbx 3 each differ primarily in their carboxy-termini and result from alternative mRNA splicing. Unlike other homeotic selector genes which are expressed transiently during development and differentiation, Pbx gene transcripts are ubiquitously expressed in both fetal and adult tissues and cell lines. Additionally, Pbx 2 and Pbx 3 transcripts are detected in lymphoid cells, which do not express Pbx 1. Pbx 4 expression is confined to the testis, especially to spermatocytes in the pachytene stage of the first meiotic prophase.

REFERENCES

1. Nourse, J., et al. 1990. Chromosomal translocation t(1;19) results in synthesis of a homeobox fusion mRNA that codes for a potential chimeric transcription factor. *Cell* 60: 535-545.
2. Kamps, M.P., et al. 1990. A new homeobox gene contributes the DNA binding domain of the t(1;19) translocation protein in pre-B ALL. *Cell* 60: 547-555.
3. Monica, K., et al. 1991. PBX2 and PBX3, new homeobox genes with extensive homology to the human proto-oncogene PBX1. *Mol. Cell. Biol.* 11: 6149-6157.
4. LeBrun, D.P., et al. 1994. Fusion with E2A alters the transcriptional properties of the homeodomain protein Pbx 1 in t(1;19) leukemias. *Oncogene* 9: 1641-1647.

PRODUCT

Pbx 1/2/3/4 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 4 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Pbx 1/2/3/4 siRNA (h): sc-43999 and Pbx 1/2/3/4 shRNA Plasmid (h): sc-43999-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

Pbx 1/2/3/4 shRNA (h) Lentiviral Particles is recommended for the inhibition of Pbx 1, Pbx 2, Pbx 3 and Pbx 4 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Pbx 1/2/3/4 (F-3): sc-28313 is recommended as a control antibody for monitoring of Pbx 1/2/3/4 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker[™] compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Pbx 1, Pbx 2, Pbx 3 and Pbx 4 gene expression knockdown using RT-PCR Primer: Pbx 1/2/3/4 (h)-PR: sc-43999-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.