SANTA CRUZ BIOTECHNOLOGY, INC.

PREP-1 siRNA (h): sc-38758

BACKGROUND

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. Fusion cDNAs have been shown to encode a protein comprised of two-thirds of the E2A transactivation domain, fused to a homeobox protein termed PRL or Pbx 1. Two highly related Pbx proteins, designated Pbx 2 and Pbx 3, have also been identified. Pbx 2 and Pbx 3 share a 92% and 94% identity, respectively, 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. Pbx-regulating protein-1, PREP-1 is a DNA-binding protein that forms stable complexes with Pbx proteins which synergize with AP-1 binding factors to augment transcription of the urokinase gene. Also referred to as UEF3, PRP-1 or p64, PREP-1 appears to be a general DNA-binding factor involved in modulating the transcriptional activity of AP-1 containing promoters.

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. Pbx 2 and Pbx 3, new homeobox genes with extensive homology to the human protooncogene Pbx 1. 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.
5. Lu, Q., et al. 1994. Fusion with E2A converts the Pbx 1 homeodomain protein into a constitutive transcriptional activator in human leukemias carrying the t(1;19) translocation. Mol. Cell. Biol. 14: 3938-3948.
6. Monica, K., et al. 1994. Transformation properties of the E2A-Pbx 1 chimeric oncoprotein: fusion with E2A is essential, but the Pbx 1 homeodomain is dispensable. Mol. Cell. Biol. 14: 8304-8314.

CHROMOSOMAL LOCATION

Genetic locus: PKNOX1 (human) mapping to 21q22.3.

PRODUCT

PREP-1 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 PREP-1 shRNA Plasmid (h): sc-38758-SH and PREP-1 shRNA (h) Lentiviral Particles: sc-38758-V as alternate gene silencing products.

For independent verification of PREP-1 (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-38758A, sc-38758B and sc-38758C.

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

PREP-1 siRNA (h) is recommended for the inhibition of PREP-1 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

PREP-1 (B-2): sc-25282 is recommended as a control antibody for monitoring of PREP-1 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 PREP-1 gene expression knockdown using RT-PCR Primer: PREP-1 (h)-PR: sc-38758-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.