

PU.1 siRNA (m): sc-36331

BACKGROUND

The Ets transcription factor family (Ets-1, Ets-2, Erg-1-3, Elk-1, Elf-1, Elf-5, NERF, PU.1, PEA3, ERM, FEV, ER81, Fli-1, TEL, Spi-B, ESE-1, ESE-3A, Net, ABT1 and ERF) are DNA-binding proteins that influence lymphoid development and activity. The Ets family monomeric proteins bind the consensus DNA site GGA(A/T) through a unique winged helix-turn-helix motif known as the Ets domain. PU.1 (Spi-1/Spi-A), Spi-B and Spi-C are closely related Ets family members which share a conserved divergent sequence within the Ets domain that enables their binding to the non-canonical AGAA sites. PU.1 transactivates a large number of B cell genes, such as those encoding CD72, CD20 and Btk, and Spi-B enhances expression of many of these same target genes. PU.1 is expressed in a wide variety of hematopoietic cells, including B cells, early T-cells, megakaryocytes, granulocytes, mast cells, immature erythrocytes and myeloid cells. Alternatively, Spi-B expression is limited to B cells and immature T cells, where expression accumulates through T-lineage commitment and then is dramatically absent following the β -selection checkpoint.

REFERENCES

1. Kola, I., et al. 1993. The Ets1 transcription factor is widely expressed during murine embryo development and is associated with mesodermal cells involved in morphogenetic processes such as organ formation. *Proc. Natl. Acad. Sci. USA* 90: 7588-7592.
2. Chen, H., et al. 1995. PU.1 (Spi-1) autoregulates its expression in myeloid cells. *Oncogene* 11: 1549-1560.
3. Chen, H.M., et al. 1995. Neutrophils and monocytes express high levels of PU.1 (Spi-1) but not Spi-B. *Blood* 85: 2918-2928.
4. Su, G.H., et al. 1996. The Ets protein Spi-B is expressed exclusively in B cells and T cells during development. *J. Exp. Med.* 184: 203-214.
5. Bemak, M., et al. 1999. Spi-C, a novel Ets protein that is temporally regulated during B lymphocyte development. *J. Biol. Chem.* 274: 10259-10267.

CHROMOSOMAL LOCATION

Genetic locus: Sfp1 (mouse) mapping to 2 E1.

PRODUCT

PU.1 siRNA (m) is a target-specific 19-25 nt siRNA designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PU.1 shRNA Plasmid (m): sc-36331-SH and PU.1 shRNA (m) Lentiviral Particles: sc-36331-V as alternate gene silencing 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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PU.1 siRNA (m) is recommended for the inhibition of PU.1 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 μ M in 66 μ l. 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

PU.1 (C-3): sc-390405 is recommended as a control antibody for monitoring of PU.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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 PU.1 gene expression knockdown using RT-PCR Primer: PU.1 (m)-PR: sc-36331-PR (20 μ l, 429 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Liu, M. and Mendicino, M. 2006. Cytokine-induced hepatic apoptosis is dependent on FGL2/fibroleukin: the role of Sp1/Sp3 and STAT1/PU.1 composite *cis* elements. *J. Immunol.* 176: 7028-7038.
2. Meng, Y., et al. 2020. MicroRNA-148a facilitates inflammatory dendritic cell differentiation and autoimmunity by targeting MAFB. *JCI Insight* 5: e133721.

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.