SANTA CRUZ BIOTECHNOLOGY, INC.

Spi-B siRNA (h): sc-37869

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 hematopoetic 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 Ets-1 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.

CHROMOSOMAL LOCATION

Genetic locus: SPIB (human) mapping to 19q13.33.

PRODUCT

Spi-B 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 Spi-B shRNA Plasmid (h): sc-37869-SH and Spi-B shRNA (h) Lentiviral Particles: sc-37869-V as alternate gene silencing products.

For independent verification of Spi-B (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-37869A, sc-37869B and sc-37869C.

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

Spi-B siRNA (h) is recommended for the inhibition of Spi-B 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

Spi-B (4G5B3): sc-517204 is recommended as a control antibody for monitoring of Spi-B 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 Spi-B gene expression knockdown using RT-PCR Primer: Spi-B (h)-PR: sc-37869-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Boyle, J.J., et al. 2012. Solid-phase immunoglobulins IgG and IgM activate macrophages with solid-phase IgM acting via a novel scavenger receptor a pathway. Am. J. Pathol. 181: 347-361.

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.