SANTA CRUZ BIOTECHNOLOGY, INC.

NFATc1 siRNA (m): sc-36054

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The Power to Question

BACKGROUND

Members of the NFAT (nuclear factor of activated T cells) family of transcription factors are related to NFκB/Rel proteins and form cooperative complexes with the AP-1 proteins, Fos and Jun, on DNA to regulate cytokine expression in T cells. NFAT proteins are widely expressed and alternatively modified to generate splice variants, and they are localized to both the cytosol (NFATc) and to the nucleus (NFATn). NFATc1 (NFATc), NFATc2 (NFATp) and NFATc3 (NFAT4, NFSTx) are predominantly expressed in immune cells, and NFAT2 and NFATc4 are expressed at high levels in cardiac tissues. In addition to activating cytokine gene transcription, NFATc2 is also implicated in cardiac valve development, and NFATc4 is involved in cardiac hypertrophy. NFAT5 is detected in both immune and nonimmune cells and, like other NFAT proteins, it contains a highly conserved Rel-like binding domain that mediates NFAT proteins associating with specific consensus sequences on DNA. NFAT proteins are activated by increases in intracellular calcium, which leads to the calmodulin-dependent phosphatase, calcineurin, dephosphorylating NFAT proteins. This activating event induces a conformational change in the protein structure that exposes the nuclear localization signal and facilitates the translocation of NFAT proteins from the cytosol into the nucleus.

CHROMOSOMAL LOCATION

Genetic locus: Nfatc1 (mouse) mapping to 18 E3.

PRODUCT

NFATc1 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 NFATc1 shRNA Plasmid (m): sc-36054-SH and NFATc1 shRNA (m) Lentiviral Particles: sc-36054-V as alternate gene silencing products.

For independent verification of NFATc1 (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-36054A, sc-36054B and sc-36054C.

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

NFATc1 siRNA (m) is recommended for the inhibition of NFATc1 expression in mouse cells.

PROTOCOLS

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

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

NFATc1 (7A6): sc-7294 is recommended as a control antibody for monitoring of NFATc1 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 NFATc1 gene expression knockdown using RT-PCR Primer: NFATc1 (m)-PR: sc-36054-PR (20 µl, 536 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Sundaram, K., et al. 2007. RANK ligand signaling modulates the matrix metalloproteinase-9 gene expression during osteoclast differentiation. Exp. Cell Res. 313: 168-178.
2. Yeo, H., et al. 2007. Cyclosporin A elicits dose-dependent biphasic effects on osteoblast differentiation and bone formation. Bone 40: 1502-1516.
3. Ogasawara, T., et al. 2013. Nanog promotes osteogenic differentiation of the mouse mesenchymal cell line C3H10T1/2 by modulating bone morphogenetic protein (BMP) signaling. J. Cell. Physiol. 228: 163-171.
4. Lee, D., et al. 2018. CCL4 enhances preosteoclast migration and its receptor CCR5 downregulation by RANKL promotes osteoclastogenesis. Cell Death Dis. 9: 495.

RESEARCH USE

For research use only, not for use in diagnostic procedures.