



NFATc3 siRNA (m): sc-36057

BACKGROUND

Members of the NFAT (nuclear factor of activated T cells) family of transcription factors are related to NF κ B/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. 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.

REFERENCES

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2. Park, J., et al. 1996. Characterization of a new isoform of the NFAT (nuclear factor of activated T cells) gene family member NFATc. *J. Biol. Chem.* 271: 20914-20921.
3. Rao, A., et al. 1997. Transcription factors of the NFAT family: regulation and function. *Annu. Rev. Immunol.* 15: 707-747.
4. Lyakh, L., et al. 1997. Expression of NFAT-family proteins in normal human T cells. *Mol. Cell. Biol.* 17: 2475-2484.
5. Ranger, A.M., et al. 1998. The transcription factor NFATc is essential for cardiac valve formation. *Nature* 392: 186-190.
6. Amasaki, Y., et al. 1998. Distinct NFAT family proteins are involved in the nuclear NFAT-DNA binding complexes from human thymocyte subsets. *J. Immunol.* 160: 2324-2333.
7. Kel, A., et al. 1999. Recognition of NFATp/AP-1 composite elements within genes induced upon the activation of immune cells. *J. Mol. Biol.* 288: 353-376.
8. Lopez-Rodriguez, C., et al. 1999. JNFAT5, a constitutively nuclear NFAT protein that does not cooperate with Fos and Jun. *Proc. Natl. Acad. Sci. USA* 96: 7214-7219.
9. Pan, M., et al. 2007. Enhanced NFATc1 nuclear occupancy causes T cell activation independent of CD28 costimulation. *J. Immunol.* 178: 4315-4321.

CHROMOSOMAL LOCATION

Genetic locus: Nfatc3 (mouse) mapping to 8 D3.

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.

PRODUCT

NFATc3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NFATc3 shRNA Plasmid (m): sc-36057-SH and NFATc3 shRNA (m) Lentiviral Particles: sc-36057-V as alternate gene silencing products.

For independent verification of NFATc3 (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-36057A, sc-36057B and sc-36057C.

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

NFATc3 siRNA (m) is recommended for the inhibition of NFATc3 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

NFATc3 (F-1): sc-8405 is recommended as a control antibody for monitoring of NFATc3 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NFATc3 gene expression knockdown using RT-PCR Primer: NFATc3 (m)-PR: sc-36057-PR (20 μ l, 474 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.