



NFATc2 siRNA (m): sc-36056

BACKGROUND

The NFAT (nuclear factor of activated T cells) family of transcription factors regulates cytokine expression in T cells. Members of the family include NFATc1 (NFATc), NFATc2 (NFATp), NFATn, NFATc3 (NFAT4, NFATx) and NFATc4 (NFAT3). Recognition of antigen by the T cell receptor (TCR) eventually activates the calcium-dependent protein phosphatase calcineurin. Once activated, calcineurin stimulates the translocation of NFATc1 (cytoplasmic) from the cytoplasm to the nucleus where it associates with NFATn (nuclear). Like NFATc1, NFATc2 resides in the cytoplasm and translocates to the nucleus subsequent to activation of calcineurin. Once in the nucleus, NFATc2 synergizes with AP-1 transcription factors to initiate transcription of cytokine genes. NFATc3 and NFATc4 share 65% sequence identity with other members of the NFAT family. They are similar to NFATc2 in that they also synergize with the AP-1 family of proteins.

REFERENCES

- Emmel, E.A., et al. 1989. Cyclosporin A specifically inhibits function of nuclear proteins involved in T cell activation. *Science* 246: 1617-1620.
- Flanagan, W.M., et al. 1991. Nuclear association of a T cell transcription factor blocked by FK-506 and Cyclosporin A. *Nature* 352: 803-807.
- Liu, J., et al. 1991. Calcineurin is a common target of Cyclophilin-Cyclosporin A and FKBP-FK506 complexes. *Cell* 66: 807-815.
- Jain, J., et al. 1993. The T cell transcription factor NFATp is a substrate for calcineurin and interacts with Fos and Jun. *Nature* 365: 352-355.
- Northrop, J.P., et al. 1994. NFAT components define a family of transcription factors targeted in T cell activation. *Nature* 369: 497-502.

CHROMOSOMAL LOCATION

Genetic locus: Nfatc2 (mouse) mapping to 2 H3.

PRODUCT

NFATc2 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 NFATc2 shRNA Plasmid (m): sc-36056-SH and NFATc2 shRNA (m) Lentiviral Particles: sc-36056-V as alternate gene silencing products.

For independent verification of NFATc2 (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-36056A, sc-36056B and sc-36056C.

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

NFATc2 siRNA (m) is recommended for the inhibition of NFATc2 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

NFATc2 (4G6-G5): sc-7296 is recommended as a control antibody for monitoring of NFATc2 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 NFATc2 gene expression knockdown using RT-PCR Primer: NFATc2 (m)-PR: sc-36056-PR (20 μ l, 582 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Chandrasekar, B., et al. 2008. Interleukin-18 suppresses adiponectin expression in 3T3-L1 adipocytes via a novel signal transduction pathway involving ERK1/2-dependent NFATc4 phosphorylation. *J. Biol. Chem.* 283: 4200-4209.
- Hwang, J.S., et al. 2015. NFAT1 and JunB cooperatively regulate IL-31 gene expression in CD4+ T cells in health and disease. *J. Immunol.* 194: 1963-1974.

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.