

NFATc4 siRNA (m): sc-38116

BACKGROUND

NFATc4 (nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 4) is a member of the nuclear factors of activated T cells DNA-binding transcription complex that influences cytokine gene expression, cardiac hypertrophy and adipocyte differentiation. This complex consists of at least two components: a cytosolic component that translocates to the nucleus upon T cell receptor (TCR) stimulation and an inducible nuclear component. Other members of this family participate in the formation of this complex. NFATc4 plays a role in the inducible expression of cytokine genes in T cells, including the induction of IL-2 and IL-4. p38 MAP kinase phosphorylates multiple residues in the NFAT homology domain of NFATc4.

REFERENCES

1. Yang, T., et al. 2001. Requirement of two NFATc4 transactivation domains for CBP potentiation. *J. Biol. Chem.* 276: 39569-39576.
2. Yang, T.T., et al. 2002. Phosphorylation of NFATc4 by p38 mitogen-activated protein kinases. *Mol. Cell. Biol.* 22: 3892-3904.
3. Wilkins, B.J., et al. 2002. Targeted disruption of NFATc3, but not NFATc4, reveals an intrinsic defect in calcineurin-mediated cardiac hypertrophic growth. *Mol. Cell. Biol.* 22: 7603-7613.
4. Graef, I.A., et al. 2003. Neurotrophins and netrins require calcineurin/NFAT signaling to stimulate outgrowth of embryonic axons. *Cell* 113: 657-670.
5. Mathew, S., et al. 2004. A ternary complex of transcription factors, NFATc4, and co-activator p300 bound to an intronic sequence, intronic regulatory element, is pivotal for the upregulation of Myosin light chain-2v gene in cardiac hypertrophy. *J. Biol. Chem.* 279: 41018-41027.

CHROMOSOMAL LOCATION

Genetic locus: Nfatc4 (mouse) mapping to 14 C3.

PRODUCT

NFATc4 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 NFATc4 shRNA Plasmid (m): sc-38116-SH and NFATc4 shRNA (m) Lentiviral Particles: sc-38116-V as alternate gene silencing products.

For independent verification of NFATc4 (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-38116A, sc-38116B and sc-38116C.

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

NFATc4 siRNA (m) is recommended for the inhibition of NFATc4 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

NFATc4 (B-2): sc-271597 is recommended as a control antibody for monitoring of NFATc4 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 NFATc4 gene expression knockdown using RT-PCR Primer: NFATc4 (m)-PR: sc-38116-PR (20 μ l, 579 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. 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.

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.