

CAML siRNA (h): sc-43659

BACKGROUND

The NFAT (nuclear factor of activated T cells) family of transcription factors regulates cytokine expression in T cells through *cis*-acting elements located in the promoters of the cytokine genes. The NFAT family consists of the cytoplasmic NFAT (NFATc), transcription factors NFATc1, NFATc2, NFATc3 and NFATc4, and nuclear NFAT (NFATn). Each of these transcription factors plays a role in T cell activation. CAML (calcium-signal modulating cyclophilin ligand) has been identified as an activator of NFAT and NF-IL2A when overexpressed in Jurkat cells. CAML has also been shown to activate calcineurin by causing calcium influx. TACI (transmembrane activator and CAML-interactor), a member of the TNF receptor superfamily, was identified based on its capacity to bind to CAML and has been shown to induce activation of NFAT in the presence of CAML.

REFERENCES

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2. Bram, R.J. and Crabtree, G.R. 1994. Calcium signalling in T cells stimulated by a cyclophilin B-binding protein. *Nature* 371: 355-358.
3. Ho, S.N., et al. 1995. NFATc3, a lymphoid-specific NFATc family member that is calcium-regulated and exhibits distinct DNA binding specificity. *J. Biol. Chem.* 270: 19898-19907.
4. Rao, A. 1995. NFATp, a cyclosporin-sensitive transcription factor implicated in cytokine gene induction. *J. Leukoc. Biol.* 57: 536-542.
5. Hoey, T., et al. 1995. Isolation of two new members of the NFAT gene family and functional characterization of the NFAT proteins. *Immunity* 2: 461-472.
6. Masuda, E.S., et al. 1995. NFATx, a novel member of the nuclear factor of activated T cells family that is expressed predominantly in the thymus. *Mol. Cell. Biol.* 15: 2697-2706.
7. Timmerman, L.A., et al. 1996. Rapid shuttling of NFAT in discrimination of Ca²⁺ signals and immunosuppression. *Nature* 383: 837-840.
8. von Bulow, G.U. and Bram, R.J. 1997. NFAT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily. *Science* 278: 138-141.

CHROMOSOMAL LOCATION

Genetic locus: CAMLG (human) mapping to 5q31.1.

PRODUCT

CAML 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CAML shRNA Plasmid (h): sc-43659-SH and CAML shRNA (h) Lentiviral Particles: sc-43659-V as alternate gene silencing products.

For independent verification of CAML (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-43659A, sc-43659B and sc-43659C.

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

CAML siRNA (h) is recommended for the inhibition of CAML 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 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

CAML (B-12): sc-166557 is recommended as a control antibody for monitoring of CAML 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 CAML gene expression knockdown using RT-PCR Primer: CAML (h)-PR: sc-43659-PR (20 μ l, 437 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.