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# CAML shRNA (m) Lentiviral Particles: sc-44438-V

## 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

1. Ho, S., et al. 1994. Cloning and characterization of NFATc and NFATp: the cytoplasmic components of NFAT. *Adv. Exp. Med. Biol.* 365: 167-173.
2. Bram, R.J., et al. 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  $Ca^{2+}$  signals and immunosuppression. *Nature* 383: 837-840.

## CHROMOSOMAL LOCATION

Genetic locus: *Caml* (mouse) mapping to 13 B1.

## PRODUCT

CAML shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see CAML siRNA (m): sc-44438 and CAML shRNA Plasmid (m): sc-44438-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^{\circ}$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^{\circ}$  C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

CAML shRNA (m) Lentiviral Particles is recommended for the inhibition of CAML expression in mouse cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CAML gene expression knockdown using RT-PCR Primer: CAML (m)-PR: sc-44438-PR (20  $\mu$ l, 389 bp). Annealing temperature for the primers should be  $55-60^{\circ}$  C and the extension temperature should be  $68-72^{\circ}$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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