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NFAT5 siRNA (h): sc-43968

BACKGROUND

Members of the NFAT (nuclear factor of activated T cells) family of transcription factors are related to NFκB/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). NFAT1, NFAT2, and NFAT4 are predominantly expressed in immune cells, and NFAT2 and NFAT3 are expressed at high levels in cardiac tissues. In addition to activating cytokine gene transcription, NFAT2 is also implicated in cardiac valve development, and NFAT3 is involved in cardiac hypertrophy. NFAT5 is detected in both immune and nonimmune cells and, like other NFAT proteins, contains a highly conserved Rel-like binding domain that mediates NFAT proteins associating with specific consensus sequences on DNA. 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

1. 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.
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.

CHROMOSOMAL LOCATION

Genetic locus: NFAT5 (human) mapping to 16q22.1.

PRODUCT

NFAT5 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NFAT5 shRNA Plasmid (h): sc-43968-SH and NFAT5 shRNA (h) Lentiviral Particles: sc-43968-V as alternate gene silencing products.

For independent verification of NFAT5 (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-43968A, sc-43968B and sc-43968C.

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 μl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μl of RNase-free water makes a 10 μM solution in a 10 μM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NFAT5 siRNA (h) is recommended for the inhibition of NFAT5 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 μM in 66 μl. 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

NFAT5 (F-9): sc-398171 is recommended as a control antibody for monitoring of NFAT5 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 NFAT5 gene expression knockdown using RT-PCR Primer: NFAT5 (h)-PR: sc-43968-PR (20 μl, 506 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Hollborn, M., et al. 2015. Regulation of the hyperosmotic induction of aquaporin 5 and VEGF in retinal pigment epithelial cells: involvement of NFAT5. *Mol. Vis.* 21: 360-377.
2. Amara, S., et al. 2016. NFAT5/STAT3 interaction mediates synergism of high salt with IL-17 towards induction of VEGF-A expression in breast cancer cells. *Oncol. Lett.* 12: 933-943.
3. Herbelet, S., et al. 2018. Localization and expression of nuclear factor of activated T-cells 5 in myoblasts exposed to pro-inflammatory cytokines or hyperosmolar stress and in biopsies from myositis patients. *Front. Physiol.* 9: 126.

RESEARCH USE

For research use only, not for use in diagnostic procedures.