SANTA CRUZ BIOTECHNOLOGY, INC.

CIITA siRNA (h2): sc-270529

BACKGROUND

The mounting of an immune response and CD4 T cell development in vertebrates require the expression of major histocompatibility complex (MHC) class II molecules. MHC class II molecules are heterodimeric cell surface glycoproteins expressed on B cells, macrophages and dendritic cells, which present antigens to $CD4^+$ T cells. CIITA (class II transactivator) acts as a coactivator for MHC class II-specific gene expression and negatively regulates the IL-4 gene promoter during T cell differentiation. IFNγ induces CIITA gene expression via JAK1 and Stat1 pathways. The GTP-binding and acidic, proline-serine-threonine-rich regions appear to be required for CIITA activity. RFX-B (also designated RFXANK and Tvl-1) is the smallest subunit of the RFX complex, which is also required for MHC class II-specific gene transcription. RFX-B contains three ankyrin-repeats that may allow protein-protein interactions between RFX-B and other RFX subunits, and possibly with CIITA and NF-Y. Defects of CIITA and RFX-B have been implicated as causes of bare lymphocyte syndrome (BLS), which is characterized by the absence of MHC class II transcription and severe immunodeficiencies.

REFERENCES

1. Steimle, V., et al. 1993. Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). Cell 75: 135-146.
2. Chin, K.C., et al. 1994. Molecular analysis of G1B and G3A IFNγ mutants reveals that defects in CIITA or RFX result in defective class II MHC and Ii gene induction. Immunity 1: 687-697.
3. Boss, J.M. 1997. Regulation of transcription of MHC class II genes. Curr. Opin. Immunol. 9: 107-113.
4. Moreno, C.S., et al. 1997. Regulatory factor X, a bare lymphocyte syndrome transcription factor, is a multimeric phosphoprotein complex. J. Immunol. 158: 5841-5848.
5. Scholl, T., et al. 1997. Specific complex formation between the type II bare lymphocyte syndrome-associated transactivators CIITA and RFX5. Proc. Natl. Acad. Sci. USA 94: 6330-6334.
6. Masternak, K., et al. 1998. A gene encoding a novel RFX-associated transactivator is mutated in the majority of MHC class II deficiency patients. Nat. Genet. 20: 273-277.

CHROMOSOMAL LOCATION

Genetic locus: CIITA (human) mapping to 16p13.13.

PRODUCT

CIITA siRNA (h2) 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 CIITA shRNA Plasmid (h2): sc-270529-SH and CIITA shRNA (h2) Lentiviral Particles: sc-270529-V as alternate gene silencing products.

For independent verification of CIITA (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270529A, sc-270529B and sc-270529C.

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

CIITA siRNA (h2) is recommended for the inhibition of CIITA 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

CIITA (7-1H): sc-13556 is recommended as a control antibody for monitoring of CIITA 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 CIITA gene expression knockdown using RT-PCR Primer: CIITA (h2)-PR: sc-270529-PR (20 µl). 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.