

ZFAT siRNA (h): sc-77821

BACKGROUND

Zinc-finger proteins contain DNA-binding domains and have a wide variety of functions, most of which encompass some form of transcriptional activation or repression. The majority of zinc-finger proteins contain a Krüppel-type DNA binding domain and a KRAB domain, which is thought to interact with KAP1, thereby recruiting histone modifying proteins. Zinc finger protein ZFAT, also known as Zinc finger protein 406, is a 1,243 amino acid protein that contains 19 C₂H₂-type zinc fingers. Single-nucleotide polymorphisms (SNPs) within the gene encoding ZFAT may be associated with susceptibility to autoimmune thyroid disease. Overexpression of ZFAT causes downregulation of many genes that are involved in the immune response. ZFAT is strongly expressed in kidney, testis, ovary, tonsil, placenta, spleen and peripheral blood leukocytes. There are three isoforms of ZFAT that are produced as a result of alternative splicing events.

REFERENCES

1. Klug, A. 1999. Zinc finger peptides for the regulation of gene expression. *J. Mol. Biol.* 293: 215-218.
2. Shirasawa, S., et al. 2004. SNPs in the promoter of a B cell-specific anti-sense transcript, SAS-ZFAT, determine susceptibility to autoimmune thyroid disease. *Hum. Mol. Genet.* 13: 2221-2231.
3. Hall, T.M. 2005. Multiple modes of RNA recognition by zinc finger proteins. *Curr. Opin. Struct. Biol.* 15: 367-373.
4. Shirasawa, S. 2006. Susceptibility genes for the development of autoimmune thyroid disease. *Nippon Rinsho* 64: 2208-2214.
5. Online Mendelian Inheritance in Man, OMIM[™]. 2007. Johns Hopkins University, Baltimore, MD. MIM Number: 610931. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
6. Koyanagi, M., et al. 2008. ZFAT expression in B and T lymphocytes and identification of ZFAT-regulated genes. *Genomics* 91: 451-457.
7. Comabella, M., et al. 2009. Genome-wide scan of 500,000 single-nucleotide polymorphisms among responders and nonresponders to interferon β therapy in multiple sclerosis. *Arch. Neurol.* 66: 972-978.

CHROMOSOMAL LOCATION

Genetic locus: ZFAT (human) mapping to 8q24.22.

PRODUCT

ZFAT 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 ZFAT shRNA Plasmid (h): sc-77821-SH and ZFAT shRNA (h) Lentiviral Particles: sc-77821-V as alternate gene silencing products.

For independent verification of ZFAT (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-77821A, sc-77821B and sc-77821C.

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

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

ZFAT (E-9): sc-398058 is recommended as a control antibody for monitoring of ZFAT 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 ZFAT gene expression knockdown using RT-PCR Primer: ZFAT (h)-PR: sc-77821-PR (20 μ l). 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.