



FAT10 siRNA (h): sc-60627

BACKGROUND

FAT10, also designated Ubiquitin D or Diubiquitin, is a 165 amino acid protein encoded in the major histocompatibility complex (MHC) that consists of two domains which share significant homology with ubiquitin. Each domain contains two cysteines, along with a free C-terminal diglycine motif required for FAT10 conjugate formation. FAT10 is inducible by interferon- γ and tumor necrosis factor α (TNF α). The FAT10 protein interacts with MAD2, a component of the spindle checkpoint, and plays a role in antigen presentation, cytokine response, apoptosis and mitosis. It may also regulate cell growth during dendritic cell or B cell activation and development. FAT10 mRNA is expressed mainly in some dendritic cells and lymphoblastoid lines and in other specific cells subsequent to interferon- γ induction. The human FAT10 gene, designated UBD, maps to chromosome 6p22.1 and is overexpressed in the tumors of various epithelial cancers.

REFERENCES

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4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606050. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
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6. Zhang, D.W., et al. 2006. p53 negatively regulates the expression of FAT10, a gene upregulated in various cancers. *Oncogene* 25: 2318-2327.
7. Ross, M.J., et al. 2006. Role of ubiquitin-like protein FAT10 in epithelial apoptosis in renal disease. *J. Am. Soc. Nephrol.* 17: 996-1004.
8. Ren, J., et al. 2006. FAT10 plays a role in the regulation of chromosomal stability. *J. Biol. Chem.* 281: 11413-11421.

CHROMOSOMAL LOCATION

Genetic locus: UBD (human) mapping to 6p22.1.

PRODUCT

FAT10 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 FAT10 shRNA Plasmid (h): sc-60627-SH and FAT10 shRNA (h) Lentiviral Particles: sc-60627-V as alternate gene silencing products.

For independent verification of FAT10 (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-60627A, sc-60627B and sc-60627C.

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

FAT10 siRNA (h) is recommended for the inhibition of FAT10 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

FAT10 (A-8): sc-393630 is recommended as a control antibody for monitoring of FAT10 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 FAT10 gene expression knockdown using RT-PCR Primer: FAT10 (h)-PR: sc-60627-PR (20 μ l, 443 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.