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GITR siRNA (h): sc-43806

BACKGROUND

The tumor necrosis factor receptor (TNFR) superfamily represents a growing family of type I transmembrane glycoproteins that are involved in various cellular functions, including proliferation, differentiation and programmed cell death. These proteins share homology for cysteine-rich repeats in the extracellular ligand binding domain and an intracellular death domain. Members of the TNFR superfamily transmit signals through protein-protein interactions, and these signals can lead to the activation of either the caspase and Jun kinase pathways, which promote cell death, or the NF κ B pathway, which results in cell survival. The glucocorticoid-induced tumor necrosis factor receptor family-related protein (GITR) is a member of the TNFR superfamily that is preferentially expressed in normal T lymphocytes from thymus, spleen and lymph nodes. GITR shares similarity with Ox40, 4-1BB and CD27 and is thought to inhibit T cell receptor-mediated cell death through the activation of the NF κ B signaling pathway.

REFERENCES

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2. Gruss, H.J., et al. 1996. Structural and biological features of the TNF receptor and TNF ligand superfamilies: interactive signals in the pathobiology of Hodgkin's disease. *Ann. Oncol.* 7: 19-26.
3. Nocentini, G., et al. 1997. A new member of the tumor necrosis factor/nerve growth factor receptor family inhibits T cell receptor-induced apoptosis. *Proc. Natl. Acad. Sci. USA* 94: 6216-6221.
4. Baker, S.J. and Reddy, E.P. 1998. Modulation of life and death by the TNF receptor superfamily. *Oncogene* 17: 3261-3270.
5. Gurney, A.L., et al. 1999. Identification of a new member of the tumor necrosis factor family and its receptor, a human ortholog of mouse GITR. *Curr. Biol.* 9: 215-218.
6. Riccardi, C., et al. 1999. Glucocorticoid hormone-induced modulation of gene expression and regulation of T-cell death: role of GITR and GILZ, two dexamethasone-induced genes. *Cell Death Differ.* 6: 1182-1189.
7. Nocentini, G., et al. 2000. Gene structure and chromosomal assignment of mouse GITR, a member of the tumor necrosis factor/nerve growth factor receptor family. *DNA Cell Biol.* 19: 205-217.

CHROMOSOMAL LOCATION

Genetic locus: TNFRSF18 (human) mapping to 1p36.33.

PRODUCT

GITR siRNA (h) is a target-specific 19-25 nt siRNA 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 GITR shRNA Plasmid (h): sc-43806-SH and GITR shRNA (h) Lentiviral Particles: sc-43806-V as alternate gene silencing products.

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

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

GITR (621): sc-53972 is recommended as a control antibody for monitoring of GITR 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 GITR gene expression knockdown using RT-PCR Primer: GITR (h)-PR: sc-43806-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.