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FOXP3 shRNA (h) Lentiviral Particles: sc-43569-V

BACKGROUND

The FOX family of transcription factors is a large group of proteins that share a common DNA binding domain termed a winged-helix or forkhead domain. During early development, FOXP1 and FOXP2 are expressed abundantly in the lung, with lower levels of expression in neural, intestinal and cardiovascular tissues, where they act as transcription repressors. FOXP1 is widely expressed in adult tissues, while neoplastic cells often exhibit a dramatic change in expression level or localization of FOXP1. The gene encoding human FOXP1 maps to chromosome 3p14.1, and the gene encoding human FOXP2 maps to chromosome 7q31. The gene encoding FOXP3, a third member of this family, maps to chromosome Xp11.23. Mutations in this gene cause IPEX, a fatal, X-linked inherited disorder characterized by immune dysregulation. The FOXP3 protein, also known as scurf, is essential for normal immune homeostasis. Specifically, FOXP3 represses transcription through a DNA binding forkhead domain, thereby regulating T cell activation.

REFERENCES

- Lai, C.S., et al. 2000. The SPCH1 region on human 7q31: genomic characterization of the critical interval and localization of translocations associated with speech and language disorder. *Am. J. Hum. Genet.* 67: 357-368.
- Banham, A.H., et al. 2001. The FOXP1 winged helix transcription factor is a novel candidate tumor suppressor gene on chromosome 3p. *Cancer Res.* 61: 8820-8829.
- Bennett, C.L., et al. 2001. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* 27: 20-21.
- Brunkow, M.E., et al. 2001. Disruption of a new forkhead/winged-helix protein, scurf, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat. Genet.* 27: 68-73.

CHROMOSOMAL LOCATION

Genetic locus: FOXP3 (human) mapping to Xp11.23.

PRODUCT

FOXP3 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see FOXP3 siRNA (h): sc-43569 and FOXP3 shRNA Plasmid (h): sc-43569-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

FOXP3 shRNA (h) Lentiviral Particles is recommended for the inhibition of FOXP3 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

FOXP3 (F-9): sc-166212 is recommended as a control antibody for monitoring of FOXP3 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor FOXP3 gene expression knockdown using RT-PCR Primer: FOXP3 (h)-PR: sc-43569-PR (20 μ l, 529 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.