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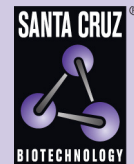
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ILT-3 shRNA (h) Lentiviral Particles: sc-44997-V

BACKGROUND

Leukocyte immunoglobulin-like receptors (ILTs, also known as LIRs) are members of the immunoglobulin superfamily of glycoproteins and are predominantly expressed by monocytes, B cells, dendritic cells, natural killer (NK) cells, peripheral blood leukocytes and tissues such as placenta, lung and liver. ILT-3 (immunoglobulin-like transcript 3), also known as HM18, LIR5, CD85K, LIR-5, LILRB5 or LILRB4, is a 484 amino acid single-pass type I membrane protein that functions as an inhibitory receptor. ILT-3 is expressed on immune cells and binds to MHC class I molecules on antigen-presenting cells. ILT-3 transduces a negative signal that inhibits stimulation of an immune response and inhibits receptor-mediated phosphorylation of cellular proteins and mobilization of intracellular calcium ions. ILT-3 contains three cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which induce inhibitory signaling cascades.

REFERENCES

1. LeMaout, J., et al. 2005. HLA-G upregulates ILT-2, ILT-3, ILT-4, and KIR2DL4 in antigen presenting cells, NK cells, and T cells. *FASEB J.* 19: 662-664.
2. Kim-Schulze, S., et al. 2006. Regulation of ILT-3 gene expression by processing of precursor transcripts in human endothelial cells. *Am. J. Transplant.* 6: 76-82.
3. Suci-Foca, N. and Cortesini, R. 2007. Central role of ILT-3 in the T suppressor cell cascade. *Cell. Immunol.* 248: 59-67.
4. Colovai, A.I., et al. 2007. Expression of inhibitory receptor ILT-3 on neoplastic B cells is associated with lymphoid tissue involvement in chronic lymphocytic leukemia. *Cytometry B Clin. Cytom.* 72: 354-362.
5. Suci-Foca, N., et al. 2007. Soluble Ig-like transcript 3 inhibits tumor allograft rejection in humanized SCID mice and T cell responses in cancer patients. *J. Immunol.* 178: 7432-7441.
6. Cortesini, R. 2007. Pancreas cancer and the role of soluble immunoglobulin-like transcript 3 (ILT-3). *JOP* 8: 697-703.

CHROMOSOMAL LOCATION

Genetic locus: LILRB4 (human) mapping to 19q13.42.

PRODUCT

ILT-3 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 ILT-3 siRNA (h): sc-44997 and ILT-3 shRNA Plasmid (h): sc-44997-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.

APPLICATIONS

ILT-3 shRNA (h) Lentiviral Particles is recommended for the inhibition of ILT-3 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

ILT-3 (H-217): sc-366213 is recommended as a control antibody for monitoring of ILT-3 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ILT-3 gene expression knockdown using RT-PCR Primer: ILT-3 (h)-PR: sc-44997-PR (20 μ l). 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.

PROTOCOLS

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