

Produktinformation



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SANTA CRUZ BIOTECHNOLOGY, INC.

CD223 shRNA (m) Lentiviral Particles: sc-44548-V



BACKGROUND

CD223 (lymphocyte activation gene-3, LAG-3) is a high affinity MHC class II ligand present on the surface of CD4+/CD8+ T cells and NK cells. CD223 shares homology in structure to CD4 molecules, having four similar extra-cellular Ig-like domains and structural motifs between D1-D3 and D2-D4 domains. CD223 has a glutamic acid-proline (EP) repetitive sequence found in other functionally distinct mammalian, parasitic and bacterial proteins that may influence a conserved biological function. CD223+/CD4+/CD8+ T cells can associate with the T cell receptor (TCR) and downregulate TCR signaling in vitro. CD223 inhibits CD4-dependent T cell function via its cytoplasmic domain. CD223 Lys 468 within a conserved "KIEELE" motif is essential for interaction with downstream signaling molecules.

REFERENCES

- 1. Demeure, C.E., et al. 2001. T lymphocytes infiltrating various tumour types express the MHC class II ligand lymphocyte activation gene-3 (LAG-3): role of LAG-3/MHC class II interactions in cell-cell contacts. Eur. J. Cancer 37: 1709-1718.
- 2. Workman, C.J., et al. 2002. Phenotypic analysis of the murine CD4-related glycoprotein, CD223 (LAG-3). Eur. J. Immunol. 32: 2255-2263.
- 3. Andreae, S., et al. 2002. Maturation and activation of dendritic cells induced by lymphocyte activation gene-3 (CD223). J. Immunol. 168: 3874-3880.
- 4. Workman, C.J., et al. 2002. Cutting edge: molecular analysis of the negative regulatory function of lymphocyte activation gene-3. J. Immunol. 169: 5392-5395.
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CHROMOSOMAL LOCATION

Genetic locus: Lag3 (mouse) mapping to 6 F2.

PRODUCT

CD223 shRNA (m) Lentiviral Particles is a pool of concentrated, transductionready 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 µl frozen stock containing 1.0 x 10⁶ 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 CD223 siRNA (m): sc-44548 and CD223 shRNA Plasmid (m): sc-44548-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

CD223 shRNA (m) Lentiviral Particles is recommended for the inhibition of CD223 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

CD223 (D-8): sc-514993 is recommended as a control antibody for monitoring of CD223 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 antimouse 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 CD223 gene expression knockdown using RT-PCR Primer: CD223 (m)-PR: sc-44548-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.