SANTA CRUZ BIOTECHNOLOGY, INC.

CD223 siRNA (h): sc-42836

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.
5. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 153337. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
6. Buisson, S., et al. 2003. MHC class II engagement by its ligand LAG-3 (CD223) leads to a distinct pattern of chemokine and chemokine receptor expression by human dendritic cells. Vaccine 21: 862-868.
7. LocusLink Report (LocusID: 3902). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: LAG3 (human) mapping to 12p13.31.

PRODUCT

CD223 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CD223 shRNA Plasmid (h): sc-42836-SH and CD223 shRNA (h) Lentiviral Particles: sc-42836-V as alternate gene silencing products.

For independent verification of CD223 (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-42836A, sc-42836B and sc-42836C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CD223 siRNA (h) is recommended for the inhibition of CD223 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 µM in 66 µl. 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

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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 CD223 gene expression knockdown using RT-PCR Primer: CD223 (h)-PR: sc-42836-PR (20 µl). 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.