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CTLA-4 siRNA (h): sc-42766

BACKGROUND

T cell proliferation and lymphokine production are triggered by occupation of the TCR by antigen, followed by a costimulatory signal that is delivered by a ligand expressed on antigen presenting cells. The B7-related cell surface proteins CD80 (B7-1) and CD86 (B7-2) are expressed on antigen presenting cells, bind the homologous T cell receptors CD28 and CTLA-4 (cytotoxic T lymphocyte-associated protein-4) and trigger costimulatory signals for optimal T cell activation. CTLA-4 shares 31% overall amino acid identity with CD28 and it has been proposed that CD28 and CTLA-4 are functionally redundant. SLAMF7 is a novel receptor on T cells that, when engaged, potentiates T cell expansion in a CD28-independent manner. B7, also designated BB1, is another ligand or counterreceptor for CD28 and CTLA-4 that is expressed on the antigen-presenting cell.

REFERENCES

- Freeman, G.J., et al. 1991. Structure, expression, and T cell costimulatory activity of the murine homologue of the human B lymphocyte activation antigen B7. *J. Exp. Med.* 174: 625-631.
- Schwartz, R.H. 1992. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell* 71: 1065-1068.
- Peach, R.J., et al. 1995. Both extracellular immunoglobulin-like domains of CD80 contain residues critical for binding T cell surface receptors CTLA-4 and CD28. *J. Biol. Chem.* 270: 21181-21187.
- Fargeas, C.A., et al. 1995. Identification of residues in the V domain of CD80 (B7-1) implicated in functional interactions with CD28 and CTLA-4. *J. Exp. Med.* 182: 667-675.
- Gribben, J.G., et al. 1995. CTLA-4 mediates antigen-specific apoptosis of human T cells. *Proc. Natl. Acad. Sci. USA* 92: 811-815.
- Cocks, B.G., et al. 1995. A novel receptor involved in T cell activation. *Nature* 376: 260-263.
- Harlan, D.M., et al. 1995. Potential roles of the B7 and CD28 receptor families in autoimmunity and immune evasion. *Clin. Immunol. Immunopathol.* 75: 99-111.

CHROMOSOMAL LOCATION

Genetic locus: CTLA4 (human) mapping to 2q33.2.

PRODUCT

CTLA-4 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CTLA-4 shRNA Plasmid (h): sc-42766-SH and CTLA-4 shRNA (h) Lentiviral Particles: sc-42766-V as alternate gene silencing products.

For independent verification of CTLA-4 (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-42766A, sc-42766B and sc-42766C.

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

CTLA-4 siRNA (h) is recommended for the inhibition of CTLA-4 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

CTLA-4 (F-8): sc-376016 is recommended as a control antibody for monitoring of CTLA-4 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 CTLA-4 gene expression knockdown using RT-PCR Primer: CTLA-4 (h)-PR: sc-42766-PR (20 μ l, 406 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Mittal, A.K., et al. 2013. Role of CTLA4 in the proliferation and survival of chronic lymphocytic leukemia. *PLoS ONE* 8: e70352.

RESEARCH USE

For research use only, not for use in diagnostic procedures.