

CD28 siRNA (h): sc-29983

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. SLAM 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

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- Körmendy, D., et al. 2013. Impact of the CTLA-4/CD28 axis on the processes of joint inflammation in rheumatoid arthritis. *Arthritis Rheum.* 65: 81-87.
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- Chen, L. and Flies, D.B. 2013. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat. Rev. Immunol.* 13: 227-242.

CHROMOSOMAL LOCATION

Genetic locus: CD28 (human) mapping to 2q33.2.

PRODUCT

CD28 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 CD28 shRNA Plasmid (h): sc-29983-SH and CD28 shRNA (h) Lentiviral Particles: sc-29983-V as alternate gene silencing products.

For independent verification of CD28 (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-29983A, sc-29983B and sc-29983C.

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

CD28 siRNA (h) is recommended for the inhibition of CD28 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

CD28 (CD28.2): sc-19655 is recommended as a control antibody for monitoring of CD28 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 CD28 gene expression knockdown using RT-PCR Primer: CD28 (h)-PR: sc-29983-PR (20 μ l). 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.