

CD154 siRNA (m): sc-29966

BACKGROUND

Resting B cells can be activated and clonally expanded into antibody-producing cells in response to a combination of cell contact and soluble signals provided by primed helper T (Th) cells. While cytokines IL-4 and IL-13 alone are inadequate for B cell activation, contact with Th cells seems to be sufficient for delivery of proliferative signals. CD40 and CD154 (also designated CD40L or TRAP) comprise a receptor ligand pair central to the transmission of this signal. CD40 is expressed on the surface of B cells and CD154 is expressed on activated T cells. In the presence of such stimulus, IL-4 and IL-13 are capable of triggering immunoglobulin class switching and secretion of IgE. CD154 is a 261 amino acid protein that is expressed as a soluble cytokine as well as a homotrimeric type II transmembrane protein. Its expression is tightly regulated, and abnormal levels of CD154 are associated with the pathogenesis of atheromatous plaque destabilization and thrombotic events. Mutations in the gene encoding for CD154 are implicated in hyper-IgM immunodeficiency syndrome type 1.

REFERENCES

1. Kehry, M.R., et al. 1994. B cell activation by helper T cell membranes. *Crit. Rev. Immunol.* 14: 221-238.
2. Hu, H.M., et al. 1994. A novel RING finger protein interacts with the cytoplasmic domain of CD40. *J. Biol. Chem.* 269: 30069-30072.
3. Rothe, M., et al. 1994. A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. *Cell* 78: 681-682.
4. Gordon, J. 1995. CD40 and its ligand: central players in B lymphocyte survival, growth and differentiation. *Blood Rev.* 9: 53-56.
5. Fuleihan, R., et al. 1995. Expression of the CD40 ligand in T lymphocytes and induction of IgE isotype switching. *Int. Arch. Allergy Immunol.* 107: 43-44.
6. Cheng, G., et al. 1995. Involvement of CRAF1, a relative of TRAF, in CD40 signaling. *Science* 267: 1494-1498.

CHROMOSOMAL LOCATION

Genetic locus: Cd40lg (mouse) mapping to X A5.

PRODUCT

CD154 siRNA (m) 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 CD154 shRNA Plasmid (m): sc-29966-SH and CD154 shRNA (m) Lentiviral Particles: sc-29966-V as alternate gene silencing products.

For independent verification of CD154 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29966A, sc-29966B and sc-29966C.

RESEARCH USE

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

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

CD154 shRNA (m) Lentiviral Particles is recommended for the inhibition of CD154 expression in mouse 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

CD154 (F-1): sc-374635 is recommended as a control antibody for monitoring of CD154 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 CD154 gene expression knockdown using RT-PCR Primer: CD154 (m)-PR: sc-29966-PR (20 μ l, 495 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.