

CD40 siRNA (m): sc-29998

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. A receptor ligand pair central to the transmission of this signal is CD40, expressed on the surface of B cells, together with CD40L, 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. B cells are sensitive to these cytokines only subsequent to CD40/CD40L-driven DNA synthesis. A downstream mediator of the CD40 signaling pathway, designated CRAF, is a member of an expanding family of proteins that contain a conserved cysteine- and histidine-rich RING finger motif. Other members of the family include TRAF1 and TRAF2. The latter proteins have been shown to regulate TNF-R2 as well as CD40 signaling through activation of the NF κ B family of transcription factors.

REFERENCES

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2. Hu, H.M., O'Rourke, K., Boguski, M.S. and Dixit, V.M. 1994. A novel RING finger protein interacts with the cytoplasmic domain of CD40. *J. Biol. Chem.* 269: 30069-30072.
3. Rothe, M., Wong, S.C., Henzel, W.J. and Goeddel, D.V. 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., Ahern, D. and Geha, R.S. 1995. Expression of the CD40 ligand in T lymphocytes and induction of IgE isotype switching. *Int. Arch. Allergy Immunol.* 107: 43-44.

CHROMOSOMAL LOCATION

Genetic locus: Cd40 (mouse) mapping to 2 H3.

PRODUCT

CD40 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 CD40 shRNA Plasmid (m): sc-29998-SH and CD40 shRNA (m) Lentiviral Particles: sc-29998-V as alternate gene silencing products.

For independent verification of CD40 (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-29998A, sc-29998B and sc-29998C.

PROTOCOLS

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

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

CD40 shRNA (m) Lentiviral Particles is recommended for the inhibition of CD40 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

CD40 (HM40-3): sc-20010 is recommended as a control antibody for monitoring of CD40 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CD40 gene expression knockdown using RT-PCR Primer: CD40 (m)-PR: sc-29998-PR (20 μ l, 478 bp). 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.