

DAP12 siRNA (h): sc-35172

BACKGROUND

Natural killer (NK) cells are regulated by stimulatory and inhibitory signals from a variety of receptors. Three main receptor families are responsible for NK cells recognition of MHC I molecules, including Ly-49, CD94/NKG2 and KIR (killer-cell inhibitory receptor). DAP12 is a phosphoprotein that is involved in the activation of NK cells. This protein interacts with membrane glycoproteins of the KIR family, resulting in cellular activation. DAP12 also binds to CD94/NKG2C, an activating NK cell receptor belonging to the C-type lectin superfamily. Additional proteins that bind to DAP12 include Ly-49D and Ly-49H, which associate with DAP12 in the plasma membrane. Phosphorylated DAP12 binds to ZAP-70 and Syk, suggesting that the activation pathway may be similar to that of the T and B cell antigen receptors.

REFERENCES

1. Lanier, L.L. 1998. NK cell receptors. *Annu. Rev. Immunol.* 16: 359-393.
2. Lanier, L.L., et al. 1998. Association of DAP12 with activating CD94/NKG2C NK cell receptors. *Immunity* 8: 693-701.
3. Smith, K.M., et al. 1998. Ly-49D and Ly-49H associate with mouse DAP12 and from activating receptors. *J. Immunol.* 161: 7-10.
4. Lanier, L.L., et al. 1998. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. *Nature* 391: 703-707.
5. Vitale, M., et al. 1998. NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis. *J. Exp. Med.* 187: 2065-2072.

CHROMOSOMAL LOCATION

Genetic locus: TYROBP (human) mapping to 19q13.12.

PRODUCT

DAP12 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 DAP12 shRNA Plasmid (h): sc-35172-SH and DAP12 shRNA (h) Lentiviral Particles: sc-35172-V as alternate gene silencing products.

For independent verification of DAP12 (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-35172A, sc-35172B and sc-35172C.

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

DAP12 siRNA (h) is recommended for the inhibition of DAP12 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

DAP12 (G-5): sc-133174 is recommended as a control antibody for monitoring of DAP12 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 DAP12 gene expression knockdown using RT-PCR Primer: DAP12 (h)-PR: sc-35172-PR (20 μ l, 419 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ohgidani, M., et al. 2014. Direct induction of ramified microglia-like cells from human monocytes: dynamic microglial dysfunction in Nasu-Hakola disease. *Sci. Rep.* 4: 4957.
2. Pereira, B.I., et al. 2020. Sestrins induce natural killer function in senescent-like CD8⁺ T cells. *Nat. Immunol.* 21: 684-694.

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.