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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

NKG2-D siRNA (h): sc-42948

BACKGROUND

The activity of natural killer (NK) cells is regulated by members of multiple receptor families that recognize class I MHC molecules, such as the killer cell inhibitory receptor/leukocyte immunoglobulin-like receptor (KIR/LIR) family and the C-type lectin superfamily. The KIR/LIR family includes p91A (also designated pp130 or PIR-B, for paired immunoglobulin-like receptor-B) and p91B (also designated PIR-A). p91A acts as an inhibitory receptor through interactions with SHP-1, whereas p91B acts as an activating receptor. CD94, NKG2 and Ly-49 are members of the C-type lectin superfamily of type II membrane glycoproteins. CD94 forms heterodimers with NKG2 isoforms on the surface of NK cells, whereas Ly-49 isoforms form homodimers. NKG2-D, expressed on NK cells, $\gamma\delta$ T cells and CD8⁺ $\alpha\beta$ T cells, is a receptor for the stress inducible protein MICA, an antigen frequently expressed in epithelial tumors.

REFERENCES

1. Long, E.O. and Wagtmann, N. 1997. Natural killer cell receptors. *Curr. Opin. Immunol.* 9: 344-350.
2. Moretta, A. and Moretta, L. 1997. HLA class I specific inhibitory receptors. *Curr. Opin. Immunol.* 9: 694-701.
3. Hayami, K., et al. 1997. Molecular cloning of a novel murine cell-surface glycoprotein homologous to killer cell inhibitory receptors. *J. Biol. Chem.* 272: 7320-7327.

CHROMOSOMAL LOCATION

Genetic locus: KLRK1 (human) mapping to 12p13.2.

PRODUCT

NKG2-D 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 NKG2-D shRNA Plasmid (h): sc-42948-SH and NKG2-D shRNA (h) Lentiviral Particles: sc-42948-V as alternate gene silencing products.

For independent verification of NKG2-D (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-42948A, sc-42948B and sc-42948C.

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

NKG2-D siRNA (h) is recommended for the inhibition of NKG2-D 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

NKG2-D (5C6): sc-53501 is recommended as a control antibody for monitoring of NKG2-D 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 NKG2-D gene expression knockdown using RT-PCR Primer: NKG2-D (h)-PR: sc-42948-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lai, H.C., et al. 2012. Activation of NK cell cytotoxicity by the natural compound 2,3-butanediol. *J. Leukoc. Biol.* 92: 807-814.
2. Lu, C.C., et al. 2014. NK cells kill mycobacteria directly by releasing perforin and granulysin. *J. Leukoc. Biol.* 96: 1119-1129.
3. Chang, C.J., et al. 2014. *Ganoderma lucidum* stimulates NK cell cytotoxicity by inducing NKG2D/NCR activation and secretion of perforin and granulysin. *Innate Immun.* 20: 301-311.
4. Lu, C.C., et al. 2016. Immunomodulatory properties of medicinal mushrooms: differential effects of water and ethanol extracts on NK cell-mediated cytotoxicity. *Innate Immun.* 22: 522-533.
5. Pereira, B.I., et al. 2020. Sestrins induce natural killer function in senescent-like CD8⁺ T cells. *Nat. Immunol.* E-published.

RESEARCH USE

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